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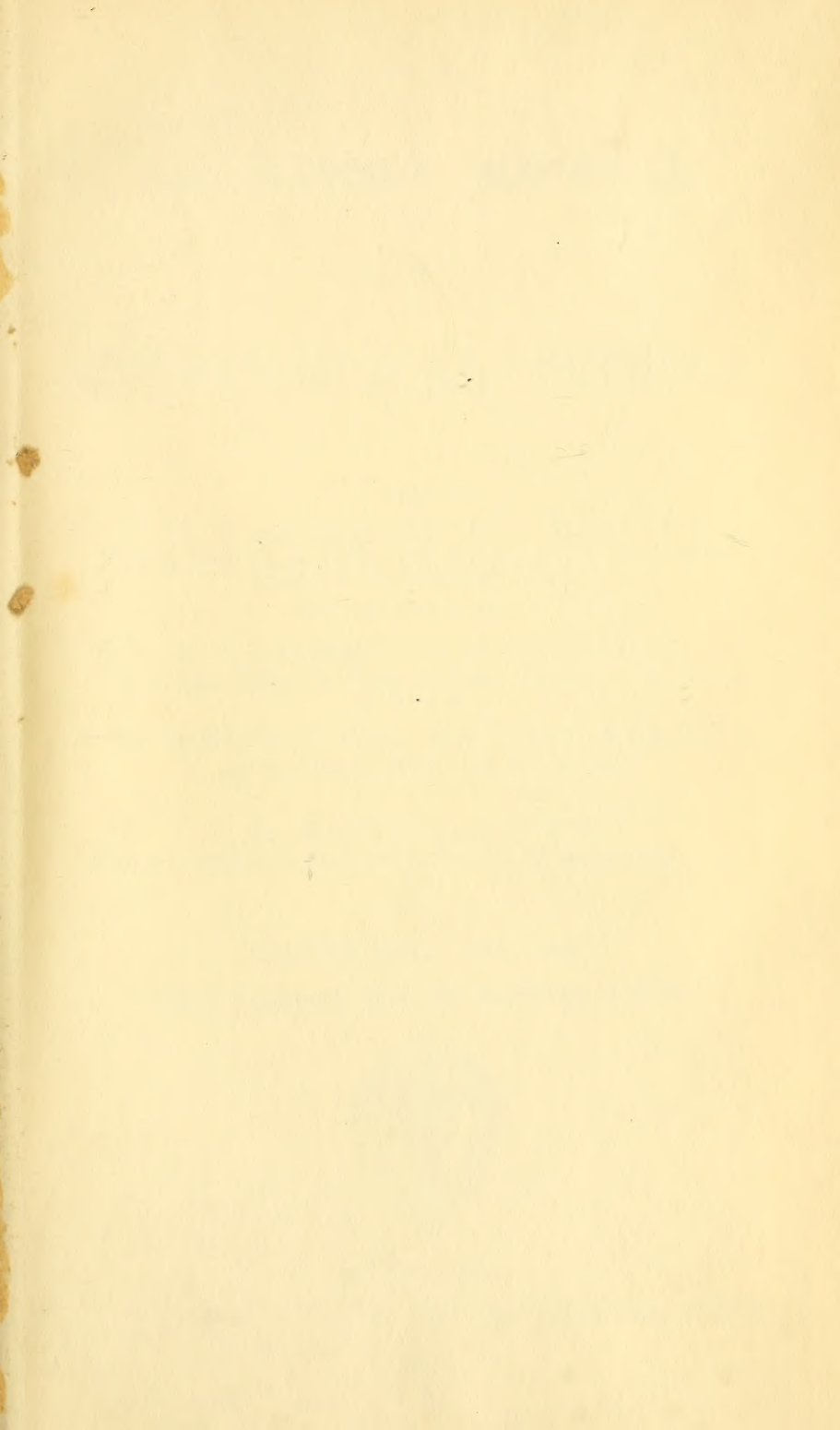
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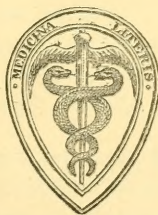
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## MEMOIRS.

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*On the MORPHOLOGY of HEMILEIA VASTATRIX, Berk. and Br. (the FUNGUS of the COFFEE DISEASE of CEYLON).* By H. MARSHALL WARD, B.A., on special duty as Cryptogamist to the Ceylon Government. (With Plates I, II, III.)

DURING the past twelve months the progress of my investigations into the life history of *Hemileia vastatrix*, the fungus so prominent in the so-called "coffee-leaf disease" of Ceylon, has resulted in the accumulation of a series of facts concerning its structure and development which have been hitherto undiscovered, or, in some cases, misunderstood. Sufficient material being now at hand to throw light upon the morphology of this remarkable parasite, I purpose bringing together shortly the main points which have been established.<sup>1</sup>

Since it is not intended to enter upon any speculations, and none of the numerous physiological and pathological phenomena of "leaf disease" can be introduced here, it appears unnecessary to discuss at length the previous publications on the subject of "coffee-leaf disease."<sup>2</sup> In so shortly referring to them no inattention is implied, but it will be impossible to clear up points of difference without numerous figures in addition to those hereto annexed. Nor is it necessary to enlarge upon the history, so far known, of this serious pest to one of our most important cultivations. I shall therefore proceed at once to the immediate object of the present paper.

<sup>1</sup> Mr. Marshall Ward has reviewed the whole course of his investigations, especially in relation to the economic aspect of the subject, and the possibility of remedial measures, in a final report to the Ceylon Government (Colombo, Sessional Paper XVII, 1881).—[Ed. 'Q. J. M. S.']

<sup>2</sup> They are chiefly—*Abbey*, 'Journ. Linn. Soc.,' Dec., 1878; *Morris*, 'Journ. Linn. Soc.,' March, 1880; and a valuable summary of the whole question up to 1880, by *W. T. Thiselton Dyer*, 'Quart. Journ. of Mic. Sci.,' April, 1880. Smaller papers by *Cooke*, *Berkeley*, *Thwaites* and *Morris*, are referred to in these.



The external appearance of a leaf severely affected with the "disease" is characterised as follows :—Small, cloudy yellow spots appear on the under side of the leaf; any one of these may be observed to increase in area and depth of colour, spreading centrifugally from a point in a more or less circular manner. Sections of such a spot show that a young mycelium is spreading in the lacunæ between the cells of the leaf, and that the discoloured area corresponds to that occupied by the mycelium. In a few days small groups of orange-coloured, granular bodies, appear externally, and, rapidly increasing in numbers, soon form an orange-red powder on the under side of the leaf; this pulverulent "rust" consists of the *spores*<sup>1</sup> developed by the internal mycelium. They arise in rosette-like groups from the stomata to which the mycelial branches have direct access from within. As age progresses the yellow colour of the "disease patch" becomes darker, and at length brown in the centre; the brown colour, which is due to destroyed leaf cells, &c., spreads centrifugally as before, and at length a shrivelled, dark-brown blotch of dead tissue is all that remains of the affected area.

This is the typical mode of development of the "disease spot," and there are several points of importance regarding it. The oldest part is always the centre, and as we proceed outwards from this, each successive phase is younger than the last. The oldest part appears to be always on the *under* side of the leaf; the discoloration of the upper side and the corresponding appearance of the mycelium there occur later. The appearance of various saprophytic fungi on the old shrivelled spot indicates the completion of the destruction. With these and other phenomena which vary the described course of the "disease spot" we are not at present concerned.

The orange-coloured "rust" consists chiefly of small, somewhat reniform bodies, which, from their structure, behaviour towards reagents, &c., and especially from their manner of germination, I have called *Uredospores*, to distinguish them from a second, less common, napiform spore, which presents sufficient analogies to the typical *Teleutospore*<sup>2</sup> of the *Uredineæ* to warrant the adoption of that name also.

The "Teleutospores" were discovered in Ceylon in March, 1880;<sup>3</sup> they occur mingled with the much more numerous *Uredospores* on the same "rust" patch, and, indeed, spring from the same spore-group. Mr. Abbay seems to have incompletely figured similar bodies, without understanding their nature,

<sup>1</sup> The evidence which proves this will be found below.

<sup>2</sup> *Vide* 'Second Report to the Ceylon Government,' 1, 1880.

<sup>3</sup> *Vide* 'Preliminary Report to Ceylon Government,' June, 1880.

on coffee from Sumatra.<sup>1</sup> Such are, shortly, the external features of the "disease spot," and we may now pass on to the details of form and structure of the parasite itself.

Since the main facts of development are now discovered, it will be perhaps the simplest plan to trace the history of the adult fungus from the *Uredospore*—to relate, in fact, what occurs after sowing the spores on coffee, giving the details of structure as we proceed.

The *Uredospore* ("Sporange" of Abbay and Morris) is figured on Plate I, fig. 1, in various positions, and is seen to be a somewhat kidney-shaped body, broader, and rounded at the free end, and slightly tapering at the other, where it is attached by a very short pedicel to the spore-bearing structure hereafter described (Pl. III, fig. 40, *e* and *f*). The free upper surface is convex from before backwards and from side to side, and is studded with small solid papillæ. The remainder of the surface forms two converging, slightly flattened sides, which gradually meet below in a broad, rounded, saddle-like ridge. This is quite smooth, concave from before backwards, and convex from side to side. The vertical transverse section of such a body is somewhat triangular, with rounded corners; but various figures are obtained by projecting the several oblique optical sections as it rolls over (Pl. I, figs. 1—3). The upper side alone is normally ornamented, though the papillæ at times occur on the upper portions of the otherwise smooth sides; these papillæ are outgrowths of the thick *exospore*, and are usually pointed and regularly distributed on its free surface (fig. 6 *d*).

The granular protoplasmic contents of the spore are enclosed by a delicate hyaline *endospore*, which becomes readily seen on germination, or may be detected by such reagents as sodic-chloride, sugar solution, &c. (figs. 6 and 7), which cause it to contract away from the *exospore*, to the inner surface of which it was before applied (fig. 4). The contents are usually coloured orange red, and at times contain oil drops of an intense orange-red tint (fig. 5); as a rule, however, the granular matrix is uniform throughout in the fresh spore. Under certain conditions the orange tinge is lost, and the contents of the spore become grey and cloudy. With these and other abnormal changes we are not here concerned. The size of these *Uredospores* averages  $\frac{1}{100}$  inch long by  $\frac{1}{100}$  broad and deep.

After lying in water for some hours it commonly happens that many of these spores become filled with spherical vacuoles, closely packed in the granular matrix, of equal or unequal size, and varying in number accordingly. A common appearance is that figured in fig. 7, and the impression of a sac filled with

<sup>1</sup> Loc. cit., Pl. 13, figs. 10, 11 and 12.

spore-like bodies is suggested. These spherical bodies are, however, not solid, but cavities excavated, so to speak, in the protoplasm, and filled with watery liquid. In the first place they are not constant, but may be seen to change their position, size, &c., very slowly; moreover, they eventually disappear, not by escaping bodily from the sac, but slowly, and this is accompanied by other changes. On crushing the whole carefully, these spheres are no longer seen in the extruded mass of protoplasm. Staining reagents do not colour them darker than the matrix, and they are not rendered clearer by such fluids as would cause contraction of denser protoplasm; on the contrary, solutions of sugar, salt, &c., and such reagents as iodine, glycerine, &c., make them disappear, evidently by the abstraction of water.

With careful treatment I have caused these vacuoles to reappear, after applying weak sugar solution, on washing in pure water. What is the nature of this vacuolisation? How far is it normal, and how far connected with other phenomena, must be left for future consideration. The proofs that these bodies are vacuoles must, however, I think, be admitted.

The strongest evidence that the papillate body is itself a spore, however, and the basis on which I have chiefly proceeded, is afforded by its germination. This occurs in pure water on glass typically as follows. At about two to five, frequently three nearly equidistant spots, the exospore becomes thinner, and pushed aside by the swelling endospore, and a delicate finger-like tube is protruded from each (fig. 8). This tube has very thin cellulose walls and a blunt, rounded apex. It may dilate slightly just beyond the place of exit, and the constriction at that spot is rendered conspicuous in contrast; otherwise the diameter of the tube is equal, and measures about one fifth of the narrow diameter of the spore (fig. 9). This germinal tube rapidly grows forwards, extending, by apical growth, as a simple unbranched cylinder for some time. As it elongates its diameter remains uniform, and its cavity continuous with that of the spore. The orange-coloured granular spore-contents meanwhile pass along the germinal tube, often presenting a most beautiful streaming motion here and there along their course; vacuoles and oil drops form both in spore and tube, and branches are soon put forth at various points, to remain short or become extended, in the same manner as before (fig. 9). This process is, however, limited, and the amount of growth is clearly dependent upon the quantity of food material originally present in the spore.<sup>1</sup>

<sup>1</sup> Attempts to grow a more extensive mycelium in nutritive fluids of various kinds have utterly failed; this is not surprising in the light of what follows.



After growing thus for some time with a sinuous course and uniform diameter the germinal tube dilates, at some place, usually near the growing end, into an ovoid or pyriform sac-like vesicle, into which all, or nearly all, the coloured contents soon pass (figs. 12 and 13), leaving the rest of the tube and spore empty of everything, except a few granules and frothy vacuoles. Fig. 11*f* shows in outline what an extensive growth may take place before this sacculation occurs; it usually occurs sooner, however.

The pyriform dilation may remain simple, or put forth branching processes here and there from any point (fig. 14). Sometimes it grows forwards as a simple tube (fig. 15), on which a similar dilatation may arise afterwards, and in this case the coloured contents pass forwards into the new vesicle. This forward growth is very rapid, and accompanied by vigorous streaming of the protoplasmic contents. It sometimes happens that a septum is formed across the neck of this swelling between it and the rest of the tube (fig. 14).

Where the branching is vigorous these vesicular bodies may become very complicated, and assume the most grotesque figures; huge vacuoles, streaming, &c., arise as the growth continues (fig. 14). This is not for long, however, and though the swellings may remain some hours after the remainder of the tube and spore have rotted, they and their contents become at length the prey of *Bacteria*, *Torulæ*, &c.

On sowing the Uredospores on the lower surface of vigorous living coffee leaves, I obtained results in the main similar. The germinal tubes produced, however, are usually shorter and less branched, or quite simple, and the whole process is apparently carried on more energetically.

At fig. 16 is represented a piece of the lower epidermis of a cotyledon of *Coffea Arabica*, on which Uredospores had been sown some eighty hours; the spores germinated and put forth the tubes freely as described above. The pyriform vesicle appears very early, and receives the whole of the contents. And now the meaning of the vesicular swelling above described becomes clearer, for it is usually formed over the orifice of a stoma and sends its processes through this into the intercellular spaces of the leaf (Pl. II, fig. 18). This is, in fact, the act of "infection." The Uredospore on germinating produces a simple mycelium (the germinal tube), which grows rapidly at the expense of the reserve material in the spore, and is only capable of further progress on reaching the interior of the leaf in this manner.

The commencement of this further development is well shown in figs. 19 and 20. That the internal mycelium is simply an

extension of the germinal tube is proved by such examples as fig. 21: the spore germinating close to the orifice of a stoma, has sent its tube directly through into the leaf without forming the preliminary vesicle. These and many similar preparations were obtained by sowing spores on living leaves kept damp in glass cells, and cutting vertical sections at periods varying from 24 to 100 hours afterwards.

I carried still further the proof of the fact that the internal mycelium is but an extension in the leaf of the germinal tube by sowing spores on the *upper* surface of leaves at places from which the epidermis had been removed; the result was a rapid growth of the germinal tube directly into the tissues, pushing its way between the palisade cells as it advanced (figs. 22 and 23). Here again no vesicle was formed. All attempts to infect by sowing on the uninjured upper surface have failed; the spores germinate, tubes and vesicular swellings form as on glass, but the whole soon shrivels and dies.

The mycelium within the leaf, then, the action and extension of which corresponds to the yellow discoloration seen externally, is clearly but a continuation of the germinal tube sent forth from the *Uredospore*, and which enters a stoma as described. Once established in the lacunæ of the leaf this soon branches, chiefly at first in the plane of the leaf, and feeding upon the products of the cells of its host, produces the injury.<sup>1</sup> At first the young mycelial tubes are very delicate, filled with fine-grained protoplasm, and somewhat stumpy; they soon become vacuolated, and more coarsely granular, and send out tufts of short, thick branches towards the cells bounding the intercellular spaces, while here and there longer "leaders" run out between the cells in various directions.

The main features of the internal mycelium thus produced are typically as follows. Its ramifications are confined to the intercellular spaces (fig. 24), except that at numerous points here and there very slender processes pierce the cell-walls to form haustoria. The mode of branching is extremely irregular, and influenced by the arrangement of the cells between which the branches run; the rate of growth, depending on several circumstances, also affects the length of the branches.

Transverse septa occur here and there, often separated by long intervals (fig. 28), especially at the peripheral parts. The diameter of a mycelial thread is about the same as that of the

<sup>1</sup> As further evidence, I may remark the success of infection experiments based on these observations. In one instance, I made sixteen separate sowings on healthy leaves of as many plants: *in fifteen cases the "disease spot" appeared where the sowing was made, and nowhere else on the plant,*

germinal tube, and, like that, generally preserves its uniform calibre throughout. In some cases the similarity between mycelium and germinal tube is rendered more striking from the contents of the former being also coloured orange-red; as a rule, however, the protoplasm of the internal mycelium is colourless. Vacuoles, granules, and oily drops occur commonly in fresh preparations (fig. 27), but in some cases the branch is filled with a dense, homogeneous protoplasm, shining with a grey, pearly lustre (fig. 26). The "coral-like" habit of the tufted, short-branched form is well seen in figs. 25 and 28.

The *haustorium* is a somewhat remarkable structure. It consists of a stiff, long neck, piercing the cell wall vertically from a branch of the mycelium (figs. 25, 29, 30); the distal end is expanded into an ovoid or pyriform body, suspended free in the cell cavity, and containing usually one or two brilliant granules surrounded by a cloudy matrix. In older specimens a distinct wall is evident. Spreading in all directions from the point of entry, the mycelial branches become applied to the exterior of the cells, and feed upon their contents by means of these haustoria, until a stage is reached when the well-nourished vegetative structure commences to form the spores, which appear externally as "rust."

This process is begun by a tuft of branches collecting in a lacuna, and growing towards the orifice of the stoma, close to which their apices remain in contact for a short time; the tightly-packed bundle then forces itself into the orifice, and pushes the common apex through to the exterior (figs. 32 and 33), where the spores are formed by budding.

The first spores arise as follows:—The spore-bearing branches, formed as above described, are filled with fine-grained grey protoplasm (fig. 33), and on reaching the exterior the apex of each expands into an ovoid sac (Pl. III, fig. 34), in which the protoplasm accumulates. A succession of other similar sacs arise by budding from the parts below this, and thus a tuft of young spores is formed (fig. 35 and fig. 40 *a, b, c*). Each of these spores arises by the protrusion of an ovoid sac, remaining narrow below, and becoming constricted off at the neck, where a septum also is formed separating the young spore from the spore-bearing branch (fig. 40). A small pedicel or stalk is usually formed, but the spore is readily detached from this, and it is rarely seen on adult specimens, though the circular place of attachment may frequently be detected.

Each spore consists at first of a simple, smooth, thin-walled sac, filled with fine-grained protoplasm, in which a nucleus-like body may be frequently observed (fig. 40, *c* and *e*). At a very early stage the existence of an endospore can be proved, but

the exospore remains smooth and thin for some time. As the regular ovoid shape of the spore becomes altered by lateral and other pressures the thickening exospore develops the solid papillæ above, and the contents begin to assume the orange-red tinge.

As the spore-bearing branches (each of which forms spores as above at first) pass through the orifice of the stoma they are crowded together into a neck: below and above this constriction they expand again. As age advances, however, they are found to become coalesced into a kind of pseudo-parenchyma, and the later-formed spores arise from the sides and top of a compound body (figs. 39 and 40 *d*), produced by their union. This structure presents the form of an oval boss, with its lower side attached by a neck, which passes through the stoma to the mycelium within the leaf; its sloping sides are covered with crowds of short, stumpy processes (fig. 41), the remains of pedicels from which spores have fallen. The fusion of mycelial elements may even extend to the internal position close to the neck, and possibly the "dark body" figured by Abbay<sup>1</sup> is this structure, into which air had penetrated.

Viewed from above, the various stages of spore formation are easily discovered. The story is the same. A few ovoid young spores arise (fig. 41 *a, b*), and additional ones bud off from near their bases (*c*), until a rosette-like cluster is formed (*e*). The appearance of the old spore-bearing head, formed by the fusion of the spore-bearing branches, is figured at fig. 41 *f*. When the "disease spot" has ceased to spread, and all, or nearly all, the spore-bearing heads have become as advanced as this, the second form of spore is produced.

This *Teleutospore* is a very remarkable body, and it is only intended here to describe its morphological characteristics. It is at first indistinguishable from the young *Uredospore*, but, instead of developing into the reniform papillate structure, it remains somewhat smaller, quite smooth, and expands into a subglobular shape. When fully formed it is napiform, and situated on a short stalk (fig. 44) attached to the spore-bearing head already described (figs. 38 and 39). An endospore is early distinguishable, and the granular protoplasmic contents soon become coloured bright orange red.

Very soon after its complete formation the central portion of the free, slightly flattened end of the oblate spheroid protrudes as a rounded, blunt, boss-like eminence; this goes on until the whole structure assumes the shape of a flask (fig. 39). This outgrowth of the apex is the first indication of germination, and the free, straight, neck-like portion is the promycelium. The

<sup>1</sup> Loc. cit., p. 177, Plate 13, fig. 4, &c.



whole process normally advances to completion, while the Teleutospore is still attached to the spore-bearing head, though detached specimens germinate quite readily in water on glass slips.

When the promycelial tube has attained a length of about six to eight times that of the Teleutospore it becomes divided by transverse septa into four subequal cells (figs. 42—45), each of which receives its share of the orange-coloured contents, which have passed along the tube from the spore. In specimens grown on glass slips under cover the promycelium and chambers are much longer (figs. 45, &c.) than those found on the leaves, and the tube may be curved and delicate in the former case, whereas in the latter the promycelium stands up stiff and straight into the moist atmosphere. This may be compared with what occurs with Uredospores germinating on glass and leaves.

The promycelium fully formed, each of the four cells (normally) sends forth a slender process, into the cavity of which the coloured contents pass (fig. 45); the process from the upper cell is simply a continuation of its apex; those from the sides of the lower cells spring from beneath the septa. The free terminal portion of each of these four outgrowths now swells into the form of a small subglobular *conidium*, which receives the remaining contents, and at last is simply attached by one point to the constricted end of the branch which produced it (figs. 42—46), and may be detached with the greatest ease.

This *conidium*, abstricted in this manner from the promycelium, is much smaller than the *Teleutospore*; it is usually subglobular, but at times subreniform or ovoid in shape, and is filled with the usual orange-red, granular protoplasm (fig. 46), enclosed in a delicate, smooth envelope. During the formation of the structures just described—*i.e.* during the later stages of its germination—the walls of the Teleutospore and promycelium become collapsed (figs. 42—46, &c.), and, being very transparent, are not easily discovered.

The *Conidia* germinate readily in water (and, indeed, even while attached to the promycelium), and may produce a long delicate tube (fig. 46 *d*) very rapidly; as a rule, however, after forming a large central vacuole, the endospore protrudes slowly (fig. 46 *a*) as a blunt, thin-walled, finger-like process, which attains a length of some four times the diameter of the conidium, and then shrivels up and dies. This very simple and transient germination is all I have been able to induce, either on glass or living leaves.

The production of Teleutospores, &c., appears to be the last act of the mycelium within the leaf, and the brown, shrivelled

remains of the "disease patch" soon becomes the prey of Bacteria, &c., which follow in the tracts of such parasites as Hemileia.

The foregoing description will naturally provoke the inquiry, to what group of fungi does Hemileia belong? Without insisting upon an answer to this question, I think it may be worth while to review some of the points in this connection. The general similarity of the "disease-spot" itself to the spots produced by many *Uredineæ* is somewhat striking, and the occurrence of the orange-red pigment in all the spore-structures, &c., vividly recalls the same. The mycelium, ramifying in the lacuna and forming tufted groups here and there externally; the septa, sometimes separated by long intervals, sometimes more closely arranged; and again, the centrifugal spread of the fungus, are all points of analogy worth recording.

It seems impossible to overlook the resemblances of the two spores of Hemileia to the *Uredospores* and *Teleutospores* of an ordinary Uredine. In size, colour, ornamentation of the exospore, mode of germination, and entry of the germinal tube through the stoma after forming a vesicular swelling over its orifice, we have strong analogies, so far as the papillate spore is concerned.

The smooth, turnip-shaped spore, in its mode and time of origin, shape, structure and colour, and especially in its germination, so strongly recalls the *Uredineæ*, that I ventured to use the well known name Teleutospore. Indeed, the promycelium, with its four cells and conidia, might almost pass for that of *Uromyces* for instance.

Nevertheless, there are some difficulties in referring Hemileia to the ordinary Uredines. The curious spore-bearing head which protrudes through the stoma, and the long-necked haustoria, so numerous on the mycelium, are perhaps the chief. How much weight these difficulties carry may be an open question. In cases where two spore-bearing heads have passed through two closely adjacent stomata, it rarely occurs that the one or two intervening cells have become destroyed; the two heads here become one common, broad, and irregular receptacle, and very like an ordinary uredinous patch.

In conclusion, it appears necessary to make a few remarks on the other forms of fungi, believed by Messrs. Abbay and Morris to be phases in the life-history of *Hemileia vastatrix*. It is impossible to explain all the points raised without numerous drawings, for which there is not room here; at a future date I hope to illustrate more fully the following brief statements.

The forms figured by Abbay<sup>1</sup> are very common in germina-

<sup>1</sup> Loc. cit., Pl. 14, figs. 10 to 19.

tion experiments out here. I recognised them at an early date, cultivated them for several months, and through several generations; they produce mycelia and forms of fructification and spores, &c., which have nothing to do with Hemileia. Their connection with the spores of the latter fungus is not genetic. So with the forms illustrated by Morris;<sup>1</sup> they belong to saprophytic or epiphyllous forms, and can in no way be genetically connected with Hemileia. Of course in such statements I am not resting on the negative evidence that no connection has been traced, but upon the results of actual cultivation of these forms, as well as the successful propagation of Hemileia itself as above described.

*May 1st, 1881.*

<sup>1</sup> 'Quart. Journ. Mic. Sci.,' April, 1880, Plates X, XI, XIII, and XIV.

*On the NATURE of the ORGAN in ADULT TELEOSTEANS and GANOIDS, which is usually regarded as the HEAD-KIDNEY or PRONEPHROS.* By F. M. BALFOUR, LL.D., F.R.S., Fellow of Trinity College, Cambridge.

WHILE working at the anatomy of *Lepidosteus* I was led to doubt the accuracy of the accepted accounts of the anterior part of the kidneys in this<sup>1</sup> and in allied species of Fishes. In order to test my doubts I first examined the structure of the kidneys in the Sturgeon (*Acipenser*), of which I fortunately had a well-preserved specimen.

The bodies usually described as the kidneys consist of two elongated bands, attached to the dorsal wall of the abdomen, and extending for the greater part of the length of the abdominal cavity. In front each of these bands first becomes considerably narrowed, and then expands and terminates in a great dilatation, which is usually called the head-kidney. Along the outer border of the hinder part of each kidney is placed a wide ureter, which ends suddenly in the narrow part of the body, some little way behind the head-kidney. To the naked eye there is no distinction in structure between the part of the so-called kidney in front of the ureter and that in the region of the ureter. Any section through the kidney in the region of the ureter suffices to show that in this part the kidney is really formed of uriniferous tubuli with numerous Malpighian bodies. Just in front, however, of the point where the ureter ends the true kidney substance rapidly thins out, and its place is taken by a peculiar tissue formed of a trabecular work filled with cells, which I shall in future call lymphatic tissue. *Thus the whole of that part of the apparent kidney in front of the ureter, including the whole of the so-called head-kidney, is simply a great mass of lymphatic tissue, and does not contain a single uriniferous tubule or Malpighian body.*

The difference in structure between the anterior and posterior parts of the so-called kidney, although not alluded to in most modern works on the kidneys, appears to have been known to Stannius, at least I so interpret a note of his in the second edition of his 'Comparative Anatomy,' p. 263, where he describes the kidney of the Sturgeon as being composed of two separate parts, viz. a spongy vascular substance (no doubt the so-called head-kidney) and a true secretory substance.

<sup>1</sup> I am about to publish in conjunction with Mr. Parker a full account of the anatomy and development of *Lepidosteus*, and shall therefore in this paper make no further allusion to it.



After arriving at the above results with reference to the Sturgeon I proceeded to the examination of the structure of the so-called head-kidney in Teleostei.

I have as yet only examined four forms, viz. the Pike (*Esox lucius*), the Smelt (*Osmerus eperlanus*), the Eel (*Anguilla anguilla*), and the Angler (*Lophius piscatorius*).

The external features of the apparent kidney of the Pike have been accurately described by Hyrtl.<sup>1</sup> He says: "The kidneys extend from the second trunk vertebra to the end of the abdominal cavity. Their anterior extremities, which have the form of transversely placed coffee beans, are united together, and lie on the anterior end of the swimming bladder. The continuation of the kidney backwards forms two small bands, separated from each other by the whole breadth of the vertebral column. They gradually, however, increase in breadth, so that about the middle of the vertebral column they unite together and form a single symmetrical, keel-shaped body," &c.

The Pike I examined was a large specimen of about 58 centimètres in length, and with an apparent kidney of about 25½ centimètres. The relations of lymphatic tissue and kidney tissue were much as in the Sturgeon. The whole of the anterior swelling, forming the so-called head-kidney, together with a considerable portion of the part immediately behind, forming not far short of half the whole length of the apparent kidney, was entirely formed of lymphatic tissue. The posterior part of the kidney was composed of true kidney substance, but even at 16 centimètres from the front end of the kidney the lymphatic tissue formed a large portion of the whole.

A rudiment of the duct of the kidney extended forwards for a short way into the lymphatic substance beyond the front part of the functional kidney.

In the Smelt (*Osmerus eperlanus*) the kidney had the typical Teleostean form, consisting of two linear bands stretching for the whole length of the body-cavity, and expanding into a great swelling in front on the level of the ductus Cuvieri, forming the so-called head-kidney. The histological examination of these bodies showed generally the same features as in the case of the Sturgeon and Pike. The posterior part was formed of the usual uriniferous tubuli and Malpighian bodies. The anterior swollen part of these bodies, and the part immediately following, were almost wholly formed of a highly vascular lymphatic tissue; but in a varying amount in different examples portions of uriniferous tubules were present, mainly, however, in the region behind the anterior swelling. In some cases I could

<sup>1</sup> "Das Uropoëtische System d. Knochenfische," Sitz. d. 'Wien. Akad., 1850.

find no tubules in the lymphatic tissue, and in all cases the number of them beyond the region of the well-developed part of the kidney was so slight, that there can be little doubt that they are functionless remnants of the anterior part of the larval kidney. Their continuation into the anterior swelling, when present, consisted of a single tube only.

In the Eel (*Anguilla anguilla*), which, however, I have not examined with the same care as the Smelt, the true excretory part of the kidney appears to be confined to the posterior portion, and to the portion immediately in front of the anus, the whole of the anterior part of each apparent kidney, which is not swollen in front, being composed of lymphatic tissue.

*Lophius piscatorius* is one of the forms which, according to Hyrtl,<sup>1</sup> is provided with a head-kidney only, *i. e.* with that part of the kidney which corresponds with the anterior swelling of the kidney of other types. For this reason I was particularly anxious to investigate the structure of its kidneys.

Each of these bodies forms a compact oval mass, with the ureter springing from its hinder extremity, situated in a forward position in the body cavity. Sections through the kidneys showed that they were throughout penetrated by uriniferous tubules, but owing to the bad state of preservation of my specimens I could not come to a decision as to the presence of Malpighian bodies. The uriniferous tubules were embedded in lymphatic tissue, similar to that which forms the anterior part of the apparent kidneys in other Teleostean types.

With reference to the structure of the Teleostean kidneys, the account given by Stannius is decidedly more correct than that of most subsequent writers. In the note already quoted he gives it as his opinion that there is a division of the kidney into the same two parts as in the Sturgeon, *viz.* into a spongy vascular part and a true secreting part; and on a subsequent page he points out the absence or poverty of the uriniferous tubules in the anterior part of the kidney in many of our native Fishes.

Prior to the discovery that the larvæ of Teleosteans and Ganoids were provided with two very distinct excretory organs, *viz.* a pronephros or head-kidney, and a mesonephros or Wolfian body, which are usually separated from each other by a more or less considerable interval, it was a matter of no very great importance to know whether the anterior part of the so-called kidney was a true excretory organ. In the present state of our knowledge the question is, however, one of considerable interest.

In the Cyclostomata and Amphibia the pronephros is a purely

<sup>1</sup> "Das Uropoetische System der Knochenfische," 'Sitz. d. Wien. Akad.' 1850.

larval organ, which either disappears or ceases to be functionally active in the adult state.

Rosenberg, to whom the earliest satisfactory investigations on the development of the Teleostean pronephros are due, stated that he had traced in the Pike (*Esox lucius*) the larval organ into the adult part of the kidney, called by Hyrtl the pronephros; and subsequent investigators have usually assumed that the so-called head-kidney of adult Teleosteans and Ganoids is the persisting larval pronephros.

We have already seen that Rosenberg was entirely mistaken on this point, in that the so-called head-kidney of the adult is not part of the true kidney. From my own studies on young Fishes I do not believe that the oldest larvæ investigated by Rosenberg were sufficiently advanced to settle the point in question; and, moreover, as Rosenberg had no reason for doubting that the so-called head-kidney of the adult was part of the excretory organ, he does not appear to have studied the histological structure of the organ which he identified with the embryonic pronephros in his oldest larva.

The facts to which I have called attention in this paper demonstrate that in the Sturgeon the larval pronephros undoubtedly undergoes atrophy before the adult stage is reached. The same is true for *Lepidosteus*, and may probably be stated for Ganoids generally.

My observations on Teleostei are clearly not sufficiently extensive to *prove* that the larval pronephros *never* persists in this group. They appear to me, however, to show that in the normal types of Teleostei the organ usually held to be the pronephros is actually nothing of the kind.

A different interpretation might no doubt be placed upon my observations on *Lophius piscatorius*, but the position of the kidney in this species appears to me to be far from affording a conclusive proof that it is homologous with the anterior swelling of the kidney of more normal Teleostei.

When, moreover, we consider that *Lophius*, and the other forms mentioned by Hyrtl as being provided with a head-kidney only, are all of them peculiarly modified and specialised types of Teleostei, it appears to me far more natural to hold that their kidney is merely the ordinary Teleostean kidney, which, like many of their other organs, has become shifted in position, than to maintain that the ordinary excretory organ present in other Teleostei has been lost, and that a larval organ has been retained, which undergoes atrophy in less specialised Teleostei.

As the question at present stands, it appears to me that the probabilities are in favour of there being no functionally active remains of the pronephros in adult Teleostei, and that in any

case the burden of proof rests with those who maintain that such remnants are to be found.

The general result of my investigations is thus to render it probable *that the pronephros, though found in the larva or embryos of almost all the Ichthyopsida, except the Elasmobranchii, is always a purely larval organ, which never constitutes an active part of the excretory system in the adult state.*

This conclusion appears to me to add probability to the view of Gegenbaur that the pronephros is the primitive excretory gland of the Chordata; and that the mesonephros or Wolffian body, by which it is replaced in existing Ichthyopsida, is phylogenetically a more recent organ.

In the preceding pages I have had frequent occasion to allude to the lymphatic tissue which has been usually mistaken for part of the excretory organ. This tissue is formed of trabecular work, like that of lymphatic glands, in the meshes of which an immense number of cells are placed, which may fairly be compared with the similarly placed cells of lymphatic glands. In the Sturgeon a considerable number of cells are found with peculiar granular nuclei, which are not found in the Teleostei. In both groups, but especially in the Teleostei, the tissue is highly vascular, and is penetrated throughout by a regular plexus of very large capillaries, which appear to have distinct walls, and which pour their blood into the posterior cardinal vein as it passes through the organ. The relation of this tissue to the lymphatic system I have not made out.

The function of the tissue is far from clear. Its great abundance, highly vascular character, and presence before the atrophy of the pronephros, appear to me to show that it cannot be merely the non-absorbed remnant of the latter organ. From its size and vascularity it probably has an important function; and from its structure this most either be the formation of lymph corpuscles or of blood corpuscles.

In structure it most resembles a lymphatic gland, though, till it has been shown to have some relation to the lymphatic system, this can go for very little.

On the whole, I am provisionally inclined to regard it as a form of lymphatic gland, these bodies being not otherwise represented in fishes.



*On the DEVELOPMENT of the SUPRARENAL BODIES in MAMMALIA.* By K. MITSUKURI, Ph.B., University of Tokio, Japan. (With Plate IV.)

It has for a long time been known that the suprarenal bodies are in close connection with the sympathetic system. As far back as 1839 Bergmann is said to have noted the fact. Remak, writing in 1847, clearly stated the relation on embryological grounds, and even went so far as to call the suprarenal bodies "*Nervendrüsen*." Leydig<sup>1</sup> is explicit in his statements in regard to the point. According to him, the suprarenal bodies in Selachians, Ganoids, and Reptiles, consist of two distinct parts. The one is enclosed, together with the usual ganglion cells, in successive sympathetic ganglia, and is made up of masses of cells different from the ganglion cells only in being dirty yellow in colour; the other part is derived from the former by the deposition of fat globules in the cells, and is situated on blood-vessels as yellowish masses. He further maintained that the ganglionic part corresponds to the medullary, and the yellowish masses to the cortical part of the Mammalian (and Avian?) suprarenal bodies, which, in these classes, are coalesced into one mass on each side. According to this view the cortical part of the suprarenal bodies of the higher types must, therefore, be derived from the medullary—a conclusion which will be shown to be erroneous. It should be mentioned that in an earlier work<sup>2</sup> Leydig did not believe in the derivation of the one part from the other—in fact, considered the yellowish masses simply as collections of fat cells.

Balfour, in his '*Monograph on the Development of Elasmobranch Fishes*,' mentions "two structures that have gone under the name of the suprarenal bodies." The one called by him the *interrenal body* is "an impaired rod-like body, lying between the dorsal aorta and the caudal vein, in the region of the posterior end of the kidneys." The other, named the *suprarenal bodies*, consists of "a series of paired bodies, situated dorsal to the cardinal veins on the branches of the aorta, and arranged segmentally." The former was believed by him to be developed from the mesoblast, and to

<sup>1</sup> '*Anatomisch-Histologisch Untersuchungen über Fische und Reptilien*,' Berlin; 1853, and '*Lehrbuch der Histologie des Menschen und der Thiere*.'

<sup>2</sup> '*Beiträge zur Mikros. Anat. &c., der Rochen und Haie*,' Leipzig, 1852.

have nothing to do with the latter, which are formed out of sympathetic ganglia, and remain in close connection with them throughout life. At that time Mr. Balfour was inclined to consider the *suprarenal bodies* homologous with the organ that goes under that name in the higher vertebrates, and the *interrenal* body, an altogether independent structure. He therefore agreed, to some extent, with the earlier view of Leydig. It will be seen that he has since modified his opinions.

Braun<sup>1</sup> studied the anatomy and development of the suprarenal bodies in Reptilians. In that class they are formed of two parts, viz. (1) masses of brown cells placed on the dorsal side of, and closely applied against, (2) irregular cords of cells, so full of oil globules that nuclei are altogether invisible in the fresh state. The first is derived from the sympathetic ganglia, and the second from the ordinary mesoblast. Here, clearly, the nervous cells, having the characteristic reaction of being stained brown by bichromate of potash, are homologous with the medullary, and the irregular cords containing oil globules, with the cortical part of the Mammalian suprarenals.

Brunn<sup>2</sup> makes an interesting statement in regard to the suprarenal bodies of birds. According to him the cells stained brown by bichromate of potash—*i. e.* the elements composing the medullary part of the mammalian suprarenals—are scattered throughout the organ between irregular cords of cells, which are like those composing the cortical part of the higher type.

Summing up these observations Mr. Balfour says, in his 'Comparative Embryology' (vol. ii, pp. 548-9), "The structure and development of what I have called the interrenal body in Elasmobranchii so closely correspond with that of the mesoblastic part of the suprarenal bodies of the Reptilia, that I have very little hesitation in regarding them as homologous; while the paired bodies in Elasmobranchii, derived from the sympathetic ganglia, clearly correspond with the part of the suprarenals of Reptilia, having a similar origin, although the anterior parts of the paired suprarenal bodies of Fishes have clearly become aborted in the higher types.

"In Elasmobranch Fishes we thus have (1) a series of

<sup>1</sup> M. Braun,—*"Bau u. Entwick. d. Nebennieren bei Reptilien," Arbeit a. d. zool.-zoot. Institut, Würzburg,* vol. v, 1879.

<sup>2</sup> A. von Brunn.—*"Ein Beitrag zur Kenntniss des feineren Baues und Entwicklungsgeschichte der Nebennieren," Archiv für mikros. Anat.,* vol. viii, 1872.

paired bodies derived from the sympathetic ganglia, and (2) an unpaired body of mesoblastic origin. In the Amniota these two bodies unite to form the compound suprarenal bodies, the two constituents of which remain, however, distinct in their development. The mesoblastic constituent appears to form the cortical part of the adult suprarenal body, and the nervous constituent the medullary part."

In view of these considerations it seemed worth while to trace the development of the suprarenal bodies in Mammalia, and see whether the medullary part is actually derived from the sympathetic system and the cortical part from the mesoblast. Accordingly, at the instance of Mr. Balfour, I have been engaged in following the history of these bodies in the rabbit, and to some extent in the rat. The result has fully justified the conclusions set forth by Mr. Balfour in the above quotations. It will be shown in this article that the medullary part of the Mammalian suprarenals arise from the sympathetic nervous system, totally independently of and outside the mesoblastic cortical part, and becomes, in the course of development, transported into the middle of the cortical part, gaining only then the position which its name implies.

Before proceeding to describe the successive stages of development it may, however, be well to recall here briefly the essential points in the structure of the adult suprarenal bodies in Mammalia. The account given below will be understood to refer to the rabbit, unless otherwise specified.

**Structure of the Adult Suprarenal Bodies.**—In the rabbit the suprarenals lie, usually in a large quantity of fat, at some distance from the kidneys, near the opening of the renal vein into the vena cava inferior, and about on a level with the anterior end of the kidney of their respective side. The right is, therefore, somewhat in front of the left, and is, moreover, on the dorsal side of the vena cava, while the left is rather on the ventral side. The suprarenals themselves are oval, reniform, or elongated in shape, and lie with their longest diameter parallel with the axis of the body. The right suprarenal is often more elongated than the left, which is generally oval or reniform, in the latter case having its concave side turned towards the median line of the body. A large vein is seen to enter each suprarenal at its posterior end, which is a branch of the renal vein in the case of the left, and of the vena cava inferior in the right suprarenal. On cutting open a fresh suprarenal, in a median longitudinal plane, its division into whitish-yellow cortical part and greyish medullary part becomes at once obvious. The

former exhibits also a subdivision into three more or less distinct layers. Starting from the outside, they are (1) a rather thin greyish layer; (2) yellowish layer comprising the main mass of the organ, and showing, even to the naked eye, radial striation; and (3) a layer, of a much darker yellow, adjoining to the medullary substance. These layers are for the most part arranged concentrically; but at the posterior end there is a modification in their relations, which, singularly enough, seems to have hitherto escaped observation. The medullary substance, instead of being covered by the cortical layers, as in other parts, here becomes attenuated into a narrow streak, and reaches the outside. Roughly speaking, therefore, the cortical substance is in the shape of a horseshoe, completely surrounding the medullary part, except at one point at the posterior end. The histological structure of this part is of great interest, as will be seen further on, on account of the peculiar developmental history of the medullary substance. A section of the suprarenal body across its shorter diameter will show three layers in concentric rings.

Fig. 1 shows a part of a transverse section of the suprarenal considerably magnified. If the whole section had been figured the outline would be oval, and the medullary substance (*m*), of which only a small portion is represented, would occupy a rather large irregularly oval area in the centre. In the figure, however, are shown all three parts of which the suprarenal is composed, viz. (1) the outer capsule of connective tissue (*d*), (2) the cortical substance (*a, b, c*), and (3) the medullary substance (*m*). I shall briefly describe each of these three portions.

Of the connective tissue capsules I need only say that nerves and blood-vessels are found embedded in it in tolerable abundance, and that bundles of connective-tissue fibres from the capsule are sent inwards to form the framework of the whole organ.

The whole space between the outer capsule and the medullary part is occupied by the cortical substance, which, therefore, constitutes the main mass of the organ. Briefly speaking, it is made up of large cells, supported in a very fine network of connective tissue—so fine that each cell has its own cavity in the mesh. These cells are collected into groups by coarser trabeculae, and the forms these cell groups assume in different layers give characteristic appearances to any particular layer. We thus recognise in the suprarenals of the rabbit three distinct zones (*a, b, c*, fig. 1), which correspond to the three layers visible to the naked eye (see above). The outer-



most zone (*a*) shows cell groups in long radiating columnar rows, which, directly under the capsule, form small arches, as shown in the figure. The cells are more closely packed than in the middle zone. Between the columns are rather large, longish spaces (*w, w*), which are shown to be sections of blood-vessels by the endothelial cells lining them. This zone gradually passes into the inner (*b*), where the columns are shorter and thicker, and cells are not as closely packed. This zone forms, in most sections, by far the largest portion of the cortical substance. Its cells stand out beautifully. Between the columns there may be seen, running radially, fine capillary blood-vessels, shown to be such by their endothelial cells. The innermost zone (*c*) has irregular cell groups, and is characterised by the presence of a great many blood-vessels (*v, v, v*). The latter, becoming larger and larger toward the centre by the union of numerous branches, finally open into the central large veins, found in the medullary substance, which, in their turn becoming one, leave the organ at its posterior end.

I may remark in passing that the two inner zones mentioned above correspond to the *zona reticularis* and *zona fasciculata* of Arnold,<sup>1</sup> but the outermost is different from his *zona glomerulosa*, as the cell groups are oval in the latter.

In sections cut with a freezing microtome from a fresh suprarenal, the whole cortical part is so filled with fat-like globules that it is hard to see anything else. These fat-like globules are not stained by osmic acid, and therefore do not seem to be true fat. In the process of hardening in picric and chromic acids and other reagents, except alcohol, they disappear completely. Even with specimens hardened in alcohol they seem to be lost in the process of embedding in wax.

The medullary cells are collected into oval or roundish groups by network of connective tissue, and I have not succeeded in seeing any one cell distinctly. The groups look like a collection of nuclei embedded in a mass of protoplasm. This alone would separate them from the cortical cells, each of which, as has been remarked, is placed in a special mesh of connective tissue, and stands out distinctly. There is, however, a reaction very characteristic of the medullary cells which is most useful in identifying them. When the suprarenals are hardened in bichromate of potash, the medullary substance is stained brown or yellow, and is sharply marked off from the cortical part. It occupies an area in the centre

<sup>1</sup> 'Virchow's Archiv,' 1866.

corresponding roughly to the outline of the section. The boundary between it and the cortical part is, however, most irregular, the latter sometimes going into the midst of the former, which, in its turn, may send long processes outwards. *In the last case we find almost invariably that the processes become continuous with nerve fibres, which frequently traverse the cortical substance from the capsule toward the medulla.*

Different authors have stated that the medullary part of various animals is very rich in ganglion cells. In the rabbit they are very scarce. Out of the numerous sections I have cut there is only one that shows undoubted nerve cells in the medulla. Large veins are found in this part (see fig. 1), and capillary spaces (*d*, fig. 2) are visible between cell groups.

Reference has been made to the posterior end of the suprarenal, where the medullary substance reaches the outside of the organ. By studying the consecutive sections of this part, we find the medullary cells following the vein to the posterior part, and finally, near the exit of the latter, emerging on the exterior. In the suprarenals of the fully-grown rabbit, the band of cells which follows the vein is rather narrow, while in those of young examples it is as wide as any part of the medullary substance. Fig. 2 is a longitudinal section of the posterior end of the suprarenal of an adult. The upper end of the figure is the posterior extremity of the suprarenal. The areas marked *c, c, c*, are occupied by cortical cells. If we follow the medullary substance (*m m*) a few sections towards the median plane of the suprarenal, we should find the point (*x*) gradually approaching the middle line of the section, and finally uniting with the main mass of the medullary substance in the centre of the organ. It will be seen in the figure that the medullary substance emerges at two points—on the top, and on the left side—and spreads itself over more or less wide areas on the surface of the suprarenal. At *f* it becomes continuous with irregular cords (*h*), made up of cell groups, which are exactly like those of the medulla, and some of which are stained yellow by bichromate of potash. They are, in fact, a part of the medulla. These cords (*h*) are continuous with the mass (*g*). This contains, in addition to small cells like those of the medullary substance, large, well-defined cells (*a*), with nuclei much larger than those of the surrounding cells. They seem to me to be nerve cells. In the sections of the corresponding part from a rabbit three weeks old I have seen an undoubted

large ganglion on one side of the section, and I am almost certain that it becomes continuous with irregular cords, continuous in their turn with the medulla. In many places, as *b*, these cords taper and seem to pass off into fibres, which are certainly like nerve fibres, and at others, as *g*, nerves pass into cell masses, and are lost among them.

Taking these facts in connection with the developmental history of the medulla, it seems to me almost certain that the process of medullary substance emerging at the outer surface of the suprarenal at its posterior end becomes in some way intimately connected with nerves and ganglion cells. I may remark that Braun (*loc. cit.*) finds ganglia at the anterior and posterior terminations of the suprarenals in the Reptiles, and that the medullary cells are in intimate connection with them. My researches appear to show that the same arrangement obtains in the rabbit at the posterior end.

In the human suprarenals, according to 'Quain's Anatomy,' the arteries are derived from the aorta, the phrenic and the renal arteries. Brunn (*loc. cit.*) remarks that many arteries entering the connective-tissue capsule divide themselves in it into numerous fine branches; some of these go directly through the cortical substance to the medulla, but by far the majority form a close anastomosis in the capsule, and enter the suprarenal with larger process of connective tissue. The fine network of capillaries in the cortical and medullary part has already been mentioned. In regard to nerves, I am only certain of the existence of one in the rabbit arising from the sympathetic cords far in front in the dorsal region, and entering the suprarenal just within its posterior half. In 'Quain's Anatomy' nerves are stated to be derived from the solar plexus of the sympathetic and from the renal plexuses, and to be exceedingly numerous.

**Development of the Suprarenal Bodies.**—According to Kölliker,<sup>1</sup> the suprarenal bodies in the rabbit appear first on the twelfth or thirteenth day of gestation as masses of somewhat large round cells on each side of, and ventral to, the aorta, on the inner side of the Wolffian bodies, and dorsal to the mesentery.

In one series of my sections of a twelve-day embryo several, that pass through what must become the front part of the future abdomen, show, at the spot designated by Kölliker, a mass of cells (*s.v.*, fig. 3) with large nuclei, stained with hæmatoxylin slightly darker than those of the surrounding cells. Dorsally this mass is tolerably distinctly separated off from

<sup>1</sup> 'Entwicklungsgesch. des Menschen und der Höheren Thiere,' p. 954.

other mesoblastic cells, but ventrally its termination is indefinite. This mass is probably the first rudiment of the mesoblastic or cortical part of a suprarenal body.

On the fourteenth day the suprarenals are already well marked. Fig. 4 shows the left suprarenal (*s. r.*) a few sections behind the front end. It consists of a mass of cell with large nuclei, divided into indefinite cords by blood capillaries which are already somewhat numerous. Cell limits are hardly visible. Mesoblastic cells surround the mass as a capsule. Dorsal to the suprarenal is placed another mass of cells (*n*) looking very much like the former, but if anything, with slightly smaller nuclei stained darker. This, on tracing it forward, is found continuous with the sympathetic cells (*symp.*, fig. 4), although at this stage the connection is not so obvious as later on.

The subsequent history shows that the medullary substance arises out of the above ventral mass (*n*) of the sympathetic system. From following the sections the suprarenal is found to be widest in the middle, and to taper both in front and behind. The cords of which it is composed are tolerably distinct, and the blood channels between them proportionally very wide and conspicuous. The sympathetic mass (*n*) gradually extends ventrally between the aorta and the suprarenal, and is continued back far beyond the posterior end of the suprarenal. It spreads, in some sections, around the latter, and in such cases it is difficult to tell the two bodies apart, as their structure at this early stage is very similar. It is only by tracing them back that they can be distinguished. The suprarenal and the sympathetic masses of the two sides remain, however, separate throughout.

In the suprarenals of sixteen-day embryos, great changes are observable. The medullary part (*m*, fig. 5) surrounded for the most part by the cortical substance (*c*) can now be clearly distinguished. In the medulla the nuclei of the cells are stained darker than in the surrounding part, and the medulla itself is at this stage nothing but a mass of simple cells with a mixture of a great number of spindle cells and other connective-tissue elements. The cortical substance is still made up of irregular cords of cells without any more definite structure. Closely applied against the suprarenal, on its inner side, there is a large mass of sympathetic nerve cells (*n*). At its ventral end, a process of this mass, partly composed of nerve fibres, enters the cortical substance at the point (*a*). The nervous mass has very much the appearance of the medullary substance—its nuclei being stained in



the same way—although there are no connective-tissue cells visible in it. On tracing it forward it becomes continuous with the main sympathetic cord dorsal to the aorta. Fig. 6 shows a section immediately behind that represented in fig. 5. Here we see the branch (*a*, fig. 5) of the nervous mass (*n*) passing into the suprarenal and uniting with the medullary substance (*m*), a relation which affords strong presumptive evidence that the latter is derived from the nervous mass without. To demonstrate this point still more conclusively I have introduced here a figure (fig. 7) of a section of the suprarenal of an embryo rat about 23 millimètres long. In this figure there are to be seen nerve fibres (*b*) starting from the nervous mass (*n*) and entering the suprarenal (*s. n*). Amongst the fibres numerous cells (*m*), that look exactly like those in the mass (*n*),<sup>1</sup> have found their way into the middle of the suprarenal, and are clearly distinguishable from the surrounding cortical cells (*c, c*) as well by the structure of the cell groups as by their darker staining. In several sections, continuous with the one figured, strands of nerve fibres dividing from the main bundle may be seen proceeding into various parts of the suprarenal, and wherever they go, they are followed by nerve cells. That these cells become the medullary substance there can be no doubt, as I have seen the parts occupied by them stained brown by bichromate of potash in somewhat older rabbit embryos. I ought to add that the mass (*n*) is continuous with the main sympathetic cords.

As we trace the sections of the sixteen-day embryo rabbits backward, we find the nervous mass (*n*, figs. 5 and 6) gradually spreading towards the ventral side of the aorta, and there constituting what must be regarded as a sympathetic plexus. This plexus seems to join the medulla of the suprarenal at more than one point. The medullary substance continues to the posterior end of the suprarenal, occupying irregular areas more or less in the centre. Towards the posterior termination it occupies by far the larger portion of a section of the suprarenal, and is covered by the cortical substance only on the outer side. The cortex finally disappears altogether, but the continuations of the medullary substance of the two suprarenals proceed backwards as paired cords along the ventral side of the aorta. These are evidently the "*Geschlechtsnerven*" of Remak, from the anterior part of which the suprarenals are stated

<sup>1</sup> The engraver has unfortunately not represented the nuclei of the mass (*n*) very satisfactorily; so that their similarity with those of the medullary substance is not obvious.

by him to be developed. These cords join the continuation of the nervous mass (*n*, figs. 5 and 6) just at the point where the cortex ceases. The cords seem to unite with each other at one point, although soon separating again. Professor Kölliker (*loc. cit.* p. 953) remarks that in the sixteen- and seventeen-day embryo rabbits, the suprarenals of two sides unite behind, while the front parts are separate. It seems evident to me that he has not clearly distinguished between the cortical and medullary part. The cortical parts of two sides certainly show no signs of uniting with each other at any stage of development, while, if reference is made only to the continuations of the medullary part, it is not only in the sixteenth to seventeenth day, but at much later stages that they are found uniting with each other. Of certain histological peculiarities of other cords I shall speak later on.

The method of entrance of the medullary substance into the suprarenal bodies may be stated briefly as follows:—The peripheral sympathetic plexus, which is formed ventrally to the aorta in the abdominal region, sends in processes into the body of suprarenals at various points—the one at the posterior end of the organ being by far the largest—and the cells thus carried in become gradually transformed into the cells of the medullary substance.

In embryos of sixteen days, all the essential parts of the suprarenals are already present, and henceforth the development consists simply in their histological elaboration:—I shall rapidly describe the successive stages until we come to the twenty-sixth day, which, being the last I have observed, I shall treat of somewhat minutely. On the eighteenth day the suprarenals are already visible to the naked eye as oval bodies on the inner side of the kidney, and substantially resemble the adult bodies in regard to position, shape, and symmetry. This stage is the earliest of which I have embryos hardened in bichromate of potash.

The medullary part takes already a slight brown staining, and it is to be specially noted that the continuation of it behind the cortical substance is also affected in the same way. The cortical substance has increased considerably in quantity, and the irregular cords of cells begin to assume a more regular oval or polygonal form. In regard to the embryos of the twentieth, twenty-second, and twenty-fourth days, of which I have sections, there is nothing special to mention, except that the suprarenals become gradually larger, that the medullary and cortical substances increase accordingly, and that the cell groups in the cortex become more and more defi-

nite and the trabeculae between them finer. We now come to the twenty-six-day embryos. Fig. 8 shows a transverse section of the left suprarenal (*s. r.*) about in the middle of the organ. The cortex (*c, c*) is already made up of definite cell groups, although not yet divided into the different zones found in the adult. The connective-tissue framework is now so fine that each cell has its own special mesh, although not so represented in the figure. Capillaries between the cell groups are well formed. The differentiated medullary substance is not so far advanced as that of the cortical part; it is divided into irregular groups of cells, whose nuclei are stained darker than those of the cortex. Its veins (*v, v*) are very conspicuous. The connective-tissue capsule is well developed.

The sympathetic nervous masses (*n, n*) are now full of distinct ganglion cells, supported in a connective-tissue network. Scattered among the larger cells are smaller cells, as at *a*. Fig. 9 shows a section of the same suprarenal near its posterior end. It will be seen that the cortex (*c, c*) no longer completely surrounds the medullary part, but is open toward the inner side. If we trace it backward we shall find it occupying less and less space in the section of the suprarenal, and gradually confining itself to the outer side. Finally, having greatly diminished in quantity, it separates from the medullary part, and soon after ends on the ventral side of the vena cava inferior. The diagrammatic longitudinal section of the same part, shown in fig. 10 A, will make the relations clear. The cortical substance (*c*) is present only on the ventral side at the posterior end of the suprarenal; and, if it were possible to show this in the same figure, would be also visible on the outside; while the medullary substance (*m.*) is confined to the dorsal and inner region. Certain parts of the medullary substance present very peculiar features. I refer to the parts marked *p, p* in fig. 9. In them, spindle and stellate connective-tissue cells are very abundant, and, by the union of their processes, seem to divide the whole space into irregularly polygonal areas. In these areas there are placed a number of small cells, somewhat like those of the central part of the medullary substance, with which the part *p, p* is directly continuous. It is the part of the medullary substance (*p, p*) which is continued posteriorly beyond the end of the suprarenal, as paired cords on the ventral side of the aorta. In fact, the part marked *p* on the left side of fig. 9 is continued from the right suprarenal, which has ended several sections in front. That this structure is really a part of the medullary sub-



stance there can be no question, as it is stained brown by bichromate of potash. It extends backwards much more than the length of the suprarenals themselves. For instance, in an embryo of twenty-four days, the right suprarenal appears in about forty sections, and the left in about thirty, while these cords extend posteriorly for about fifty-five sections from the termination of the left suprarenal. The cords unite with each other several times, soon, however, separating again. Posteriorly, they gradually lose their brown colour, and seem then made up of nerve fibres. Nerves may also join them, where they still exhibit the typical brown reaction. It is probable, from the comparison of the sections of embryos hardened in picric acid and potassium bichromate, that so long as the histological structure shown in *p*, fig. 9, is observable, the cords are stained brown. I have carefully traced the cords forward both in the longitudinal and transverse sections, and find that they become continuous with the nervous mass (*n*) shown in fig. 8. This is obvious from the series of sections in fig. 10. A is the outermost, the sections gradually going towards the median axis of the body of the embryo. In A the cortex (*c*) is quite large, while the part marked *p* (same as *p*, fig. 9) is continuous with the medullary substance (*m*). The nervous mass (*n*, of fig. 8) is here also marked *n*. In B the cortex (*c*) is disappearing; in C it has completely disappeared, and the part marked *p* has divided into two parts (*p* and *a*). Lastly, in D, we see that the nervous mass (*n*) has united with the small mass marked *a* in C. Thus there can be no doubt that the structure *p*, figs. 9 and 10, is continuous with the nervous mass *n*, fig. 8, and must be of a nervous nature. And yet it has not a single typical ganglion cell, and there is a considerable difference between its microscopic appearance and that of the nervous mass (*n*, figs. 8, 9, and 10). It is possible that the cells that are found in *p* are of the same nature as the smaller cells in the nervous mass. The only conclusion that I can arrive at is that this part of the peripheral sympathetic system becomes early distinguished from the other parts by an enormous development of connective-tissue cells, and by a total absence of ganglion cells, and that all this is preparatory to its transformation into the medullary substance of the suprarenal. The irregular polygonal areas, in which the cells are embedded, are not unlike the cell groups of the medullary substance, and might be easily transformed into them. This, taken in connection with the facts that this structure (*p*, *p*, figs. 9 and 10) is directly continuous with the medullary substance, and seems gradually



to pass into it, and that it is stained by bichromate of potash seems to justify this conclusion. Its posterior extension presents no objection to this view, as we have seen that in the adult suprarenal the medullary substance is found outside the organ in a corresponding region.

The results at which I have arrived in the preceding pages may be summed up as follows :

(1) The suprarenal bodies in Mammalia are composed of two parts—the cortical and the medullary—totally different in their origin.

(2) The cortical substance arises from the mesoblast.

(3) The medullary substance is derived from the peripheral part of the sympathetic system, and is at first placed outside of the cortical substance, becoming transported into the middle of the suprarenal body in the course of development.

I may also call attention once more to the interesting gradation of the structure of the suprarenals in different groups of Vertebrata. In Elasmobranchs the two components of the suprarenals are totally independent of each other. The sympathetic part is found in successive sympathetic ganglia, while the mesoblastic part is a median unpaired body. In Reptilia the sympathetic part is no longer bound up in sympathetic ganglia, but is closely applied against the dorsal side of the mesoblastic part, which is paired. In Aves the sympathetic part has found its way inside of the mesoblastic part, but is as yet irregularly scattered throughout the organ (Brunn). Finally, in Mammalia the sympathetic part is collected into one mass, and occupies an area in the centre of the mesoblastic part, although still showing its origin in its development.

In conclusion, I wish to express my sincerest obligations to Mr. Balfour, with whom the idea of the present investigation originated, for his uniform kindness in giving me his valuable advice, and also for the facilities which he has afforded me in his laboratory for pursuing my work.

OBSERVATIONS *on the* RESTING STATE *of* CHLAMYDOMYXA LABYRINTHULOIDES, Archer. By PATRICK GEDDES, F.R.S.E., Lecturer on Zoology in the School of Medicine, Edinburgh, and Demonstrator of Botany in the University. (With Plate V.)

I RECENTLY received from Professor Dickson a plant of *Sphagnum*, forwarded by Professor Perceval Wright, from Westminster, and laden with specimens of *Chlamydomyxa labyrinthuloides*, Archer, a remarkable organism upon which nothing further has been written since the paper by its discoverer, which appeared in this Journal in 1875.<sup>1</sup> I have not had the good fortune to witness the remarkable motile labyrinthuloid state, so fully described and beautifully figured by Mr. Archer; this, however, is not to be wondered at, since he mentions that even in the natural conditions, its occurrence is occasional and capricious. My attention has therefore been confined to the resting state, on which I have been able to confirm, and have attempted somewhat to extend, his observations.

From such average adult resting forms as those figured by Mr. Archer, or again at Pl. V, fig. 13, of the present paper, there are to be found absolutely perfect gradations to such forms as those represented at figs. 1 *a* and 2. These are spherical, with their cellular walls filled with protoplasm uniformly coloured with chlorophyll, save usually for one bright red speck. They are usually alone, but are occasionally grouped, generally in twos or fours. Their resemblance to *Protococcus pluvialis* is extremely striking, in fact it would be easy to place specimens under the microscope which would be necessarily so termed by every observer unacquainted with *Chlamydomyxa*. It is only by their extreme variability, by the existence of every possible gradation from these forms downwards to others much smaller than ordinary *Protococci*, and upwards to the massive and irregular bulk of a full-grown *Chlamydomyxa* (which is quite visible to the naked eye), that one is enabled to recognise that these are simply young specimens of the latter in what we may henceforth term the *Protococcus* stage.

As growth goes on, the little mass, usually enclosed within a cell of the *Sphagnum* leaf, remains spherical until it touches its prison wall, when—one diameter being henceforth retained—it proceeds to grow lengthwise, proceeding often for a considerable

<sup>1</sup> 'Quart. Journ. Mic. Sci.,' vol. XV, 1875, Pl. VI, "On a New Sarcodic Organism," &c.

distance, forming a more or less elongated, annulated mass (fig. 1 *c*), but sooner or later bursts through its prison wall, forming a more or less spheroidal droplet of protoplasm on the outer surface of the leaf (fig. 1 *d, e*). Here growth goes on to a great extent, the volume of protoplasm outside the leaf far exceeding, in adult specimens, that contained within; while frequently the growth of the outer portion is still further assisted by the passage of the whole or part of the protoplasm from the interior to the exterior, either at once (fig. 20) or at successive times (figs. 12, 21, 22), each recession of the protoplasm from the first-formed wall continuous with that of the *Sphagnum* cell being in the latter case very clearly marked by a corresponding wall of cellulose. However, whether the protoplasm passes entirely to the exterior or not, the organism remains firmly held in its place by the continuity of the internal and older portion of the cellulose wall with the new investment, and a great number of specimens remain thus attached throughout life (figs. 1 *f*, 21, 22), as can be seen by placing an entire leaf of *Sphagnum* under the microscope, although when the specimens are removed by the aid of a needle the wall of course breaks at the narrow neck which connects the outer and inner portions of the wall, and their mode of attachment is thus apt to escape notice.

The outer mass may either simply enlarge more or less regularly, or if a long period of rest has intervened, and a cellulose wall of great thickness and homogeneity has consequently been formed, a new departure may take place, the wall giving way at the weakest place, and a new outflow issuing forth. Such a mode of growth, of course, gives rise to an extraordinary variety of forms, the new outflow, apparently incipient in fig. 20, being sometimes larger (fig. 1 *f*), sometimes smaller (fig. 8), than the previous one; sometimes, too, so nearly equal in size as to produce forms like figs. 9 *a*, 9 *b*. The newer mass usually remains completely continuous with that of which it is an extension, but sometimes is attached by a mere thread of protoplasm (fig. 8) or of cellulose (fig. 9 *b*), or may no doubt sometimes also become detached altogether, as seems to have been the case with one or both of the two smaller masses in fig. 8.

But while waste is continuous throughout life, growth is more or less irregular, and size thus tends to be diminished. Sometimes the protoplasm shrinks within its wall, and fits it loosely with a long-encysted amoeba; more frequently, however, after recession to a certain distance a new cyst is thrown out as in fig. 22 or fig. 13, the latter of which exhibits an interesting alteration of growth and diminution.

In the process of shrinking, a morsel of protoplasm, adherent, perhaps, to the adjacent cell wall, or confined by the neck which

separates a new outflow from the parent mass, often becomes separated off, and encysts itself independently (fig. 13). From this to such forms as those represented in figs. 14, 15, 16, the transition is easy. The new cysts frequently, but by no means universally, contain a portion of the red colouring matter so largely possessed by this organism, their size may be approximately equal (fig. 14), or extremely irregular (fig. 16), and the number may vary from two up to a dozen or more. Such forms as those represented in figs. 6 *a*, 6 *b*, where two equal or similar cysts are formed, are rare; still more so cases where a transverse septum is formed without encystment (figs. 7 *a*, 7 *b*). The young cysts appear usually to be liberated by disintegration of the enclosing wall of the parent mass, but sometimes also recommence activity without this completely taking place as in fig. 17, where one appears in active growth within the old wall, while the others (two of which are closely united with the parent wall) are empty. Small, flat, amœboid masses, almost naked, or enclosed in very thin walls (fig. 10), occasionally occur on the surface of the leaf; these are probably organisms which have escaped from their cyst, and which, after a period of wandering, are returning to the resting state.

The new cysts, variable though they are, are quite distinguishable from the *Protococcus* forms first described, and these latter certainly do not arise in this way. At what particular juncture in growth the labyrinthoid streaming takes place I cannot, of course, say, yet am inclined to suspect that the *Protococcus*-like germs may arise simply from detached fragments of the moving network.

When the *Protococcus* forms are not enclosed within the cells of the *Sphagnum*, a considerable size is occasionally reached before the spheroidal form is lost (fig. 3). I have observed instances, both inside and outside the *Sphagnum* leaf, of division into two and even four masses (figs. 1*a*, 4, 5 *a*, *b*), a phenomenon of considerable interest, since the resemblance to *Protococcus* is rendered much closer. The resemblance, too, of such conditions, particularly of those represented in figs. 5 and 6, to *Glæocapsa*, even in the disposition of the laminæ of cellulose, is even more striking.

In the search for a nucleus, I have been no more successful than Mr. Archer. Larger masses of protoplasm, generally impregnated with red colouring matter, are very frequently present, and occasionally one finds peculiar bodies which resemble a nucleus, sometimes even with contained nucleolus (figs. 19 *a*, 19 *b*, 21, 23 *b*); these, however, are rather to be regarded as smaller secondary cysts, formed in exceptional circumstances within the general mass of protoplasm.



A yellowish colouring matter, presumably xanthophyll, is often associated with the chlorophyll. The disposition of the chlorophyll, usually quite diffuse or irregular, is occasionally in comparatively definite patches (fig. 11 *a, b*), placed at tolerably regular intervals in the protoplasm. It is impossible to avoid the suggestion that these are incipient forms of the definite chlorophyll granules of higher plants. The red colouring matter occurs with great, though not perfect constancy. It appears to arise from the change of green colouring matter, as is shown by treatment with reagents, when the red often slowly dissolves out, leaving a yellowish green mass of protoplasm. The red colouring matter is sometimes produced in such abundance as to give the *Sphagnum* leaves a ruddy appearance to the naked eye. The most curious circumstances connected with this red material, and one to which, so far as I am aware, there is no parallel, is, that in a decided majority of the adult cysts which I have examined, a greater or less quantity of red colouring matter, in addition to that which is normally present free in the cell contents, is being stored away in tiny drops or granules under successive laminæ of cellulose, and thus curious compound warts, often projecting far into the interior of the cyst, are formed. These can be best studied by compressing the preparation so as to burst the cyst and drive out the protoplasmic contents. These warts are very rare in the smallest specimens, such as fig. 23 *b*, but are of usual occurrence in the largest. They sometimes commence developing quite early in the history of the cyst, and their component laminæ are then continuous with those deposited over the whole interior (fig. 14), while at other times their development does not commence until after all the laminæ have been laid down (fig. 23 *a*).

The curious form represented at fig. 24 has, in addition to two large and well-laminated warts, a curious central pillar. This has evidently been produced by the young mass surrounding at the moment of outflow a morsel of conferva, which, notwithstanding the thick deposit of cellulose covering it, appears to have continued in life, since its contents are still remaining.

That the separate laminæ of cellulose in such specimens as those figured at figs. 12, 13, 21, 22 of the plate, has been successfully deposited, the youngest innermost, is beyond all doubt. But these laminæ are only separate for a short distance, and soon unite to form the cell wall for the rest of the circumference. The lamination, though, is in most cases apparent, is in some by no means so; it can usually be brought out by treatment with solution of potash. Here, then, is a distinct case of a cell wall distinctly formed by the deposition of successive laminæ, not by intussusception and subsequent differentiation; an important

fact in view of the wide prevalence of the latter and somewhat overstrained theory.

But the principal interest of these observations lies in the fact that here we have living protoplasm assuming the most protean forms, despite the cellulose wall; so much so, that the plate might seem rather to include a whole series of organisms than to be devoted to the resting state of a single species. Without insisting on the consideration that such extreme variability leads one to look with greatly increased suspicion upon the type-figures of many genera and species of amœboid organisms, it is worthy of notice that here we have represented by the same organism, if not all the modes of cell multiplication with which we are acquainted, at any rate processes which closely resemble these, and which assist us in imagining how they have arisen. Thus, figs. 4, 5, and 7 closely simulate the ordinary process of transverse division; figs. 1 and 8 show us gemmation; while fig. 14 and its neighbours, are cases, apparently, at least, akin to those of free cell formation; while figs. 10 and 17 indicate the probable existence of rejuvenescence. We have, in short, here an organism even better adapted than the *Amœba* to serve as a type of the most undifferentiated cell; its cellulose wall keeps a permanent record of every change, its variability is so great that one can feel a sort of personal interest in drawing every specimen, and can recognise it again among a thousand, while its extreme plasticity and sensitiveness to every change in the environment suggest that by changing the plant upon which it lives, it would be easy to impress upon it other forms, to run it into different moulds.

What are the affinities, and what should be the systematic position of so protean an organism? Mr. Archer's reference to the *Labyrinthulidea* can scarcely suffice us, even if a question did not immediately arise as to their position and affinities. Its semi-amœboid character in the resting stage, and its exalted amœboid activity when motile, might tempt one rather to refer it to the *Thalamophora*. Its cellulose wall, its red, green, and yellow colouring matter makes it seem rather referable to the *Algæ*, a view greatly strengthened by the existence of a *Protococcus* stage, while, as my friend Mr. Macfarlane suggests, it would thus take the place among the lower *Algæ* which the *Myxomycetes* do among the lower fungi. On the whole I am inclined to regard it as a degenerate form from the *Palmellaceous Algæ*, but one sufficiently aberrant to take place alone, and form the type of a new order, the *Chlamydomyxida*. In any case, it is almost an ideal "Protist," and cannot be distinctly appropriated by either botanist or zoologist without a certain violence to the other.

REVIEW of RECENT RESEARCHES on KARYOKINESIS<sup>1</sup> and CELL DIVISION. By J. T. CUNNINGHAM, B.A., Scholar of Baliol College, Oxford. (With Plate VI.)

THE following is a short account of the latest investigations into the metamorphosis which nuclei undergo in the process of indirect division, and of the present state of knowledge and speculation concerning the structure of the cell and the phenomena of its life.<sup>2</sup>

**Methods of Examination.**—The structure of the nucleus and cell, and the forms which the elements of the nucleus assume during division, can only be made out clearly in preparations from tissues which have been fixed and stained. The reagents which have been found most valuable for other histological researches are not always to be relied on for the demonstration of karyokinetic figures. Strasburger fixed his vegetable tissues by placing them, when quite fresh, in absolute alcohol. Others have used the salts of chromic acid, which are so useful for the isolation and definition of cells. Klein<sup>3</sup> states that he has found chromate of ammonia especially successful. Flemming, on the other hand, believes that all fixing reagents are more or less untrustworthy, except chromic acid, 1 per cent. solution, or saturated solution of picric acid. Alcohol and acetic acid usually produce alterations, but the latter is useful in observations on fresh tissues; and Peremeschko and Flemming have obtained good results by treating fresh objects with acetic acid and Bismarck brown. For staining permanent preparations various dyes may be employed; hæmatoxylin is one of the most certain, but Hermann's aniline fluid, methyl-green, safranin, and various carmine dyes, such as alum-carmine, borax-carmine, are valuable in many cases. In the study of the ova of Echinoderms Fol and Flemming obtained good preparations with osmic acid and carmine, but most of Flemming's<sup>4</sup> new

<sup>1</sup> This term was first used by Schleicher ('Die Knorpel-zelltheilung, Arc. mik. Anat.,' Bd. 16) to denote the stages in the process of division which precede the formation of the equatorial plate; but it is now agreed that it shall include the whole metamorphosis from the resting state of the mother-cell to the return to that state in the daughter-cells (*κάρνον*, nucleus; and *κίνησις*, movement).

<sup>2</sup> See Mr. Priestley's *résumé* in this Journal, April, 1876.

<sup>3</sup> This Journal, April, 1879.

<sup>4</sup> "Beiträge zur Kenntniss der Zelle," &c., iii Theil. 'Archiv Mik. Anat.,' Bd. xx, 1881.



results were gained by colouring fresh eggs on the slide with saffranin or some aniline dye, and then adding acetic acid, or by using the compound acetic acid carmine, after Schneider's method. Sometimes Flemming found that the acetic acid carmine gave a better staining when the ova had previously been treated with nitric acid of 40 or 50 per cent. strength, and then carefully washed with distilled water.

Concerning the main features of the process of karyokinesis there is a striking similarity in the descriptions of the numerous observers, although the objects of observation differ so widely, but there is great variety of opinion as to the details. Some of the discrepancies will probably vanish with increase of knowledge, but the process doubtlessly varies to some extent with the character of the cell.

**Flemming's Typical Case.**—According to Flemming,<sup>1</sup> who took for the chief material of his careful researches the tissues of *Salamandra*, the changes which take place in the indirect division of a cell are those represented in figs. 1—15. The nucleus in its resting state is enclosed by a membrane which, in optical section, shows a double contour (fig. 1). Within the membrane is a "reticulum" or framework (*Gerüst*) of fibrils, a homogeneous ground substance, one or more nucleoli, and in some cases a few smaller granules. Flemming believes the nucleoli to be connected with the fibrils of the reticulum, and denies that there are any independent granules; but the majority of investigators speak of nucleoli and granules lying free in the ground substance. In the resting nucleus all the elements are stained by dyes—the membrane, the nucleoli, the fibrils, and the ground substance. When the nucleus is about to divide the membrane disappears, together with the nucleoli and all granules and thickenings of the fibrils. The mass of fibrils loses its reticular character through the disappearance of the nodal points, which Klein and Flemming believe to be in great part the cause of the granular character so often ascribed to nuclei. There are no free ends of fibrils to be seen, but an appearance such as would be presented by long endless fibrils bent irregularly in all directions. This stage is called by Klein<sup>2</sup> that of the "convolution," which seems to be the only short equivalent in English of the expressive German word "*Knäuel*." In this and all succeeding stages of the metamorphosis only the fibrils are affected by dyes; the ground substance remains transparent, and round the mass of fibrils is a clear space

<sup>1</sup> 'Virchow's Archiv,' Bd. 77, 1879.

<sup>2</sup> 'Atlas of Histology,' 1880.



separating it from the cell substance. Flemming concludes from this that the nucleus is composed of two substances, of which one is stained by dyes the other not, and he accordingly calls the former chromatin, the latter achromatin. He believes that both of these substances are contained in each element of the resting nucleus, while at the beginning of karyokinesis all the chromatin is converted into fibrils, the achromatin filling up the interstices between these and forming the clear space round the fibrillar mass.

The convolution, by a change in the arrangement of the fibrils, passes into the "wreath" form (fig. 3), the bends being arranged, with more or less regularity, round a central space. After this the mass of fibrils, in which no free ends have hitherto been visible, breaks up into a number of V-shaped loops, each with a bend and two diverging limbs. These arrange themselves with the bends placed centrally and the limbs directed peripherally, forming a star (mother star). Each of these loops, after it is formed divides itself, according to Flemming, into two by a longitudinal splitting of the fibril (fig. 5); but this statement is not accepted by Klein (loc. cit.), who was unable to determine with the highest powers whether the appearance referred to was due to a longitudinal splitting or caused by the fibrils becoming tubular. Flemming<sup>1</sup> says that owing to this splitting the loops become twice as numerous and only half as thick, and that since the loops again become less numerous and thicker after the formation of the daughter stars, a fusion of the loops in pairs must occur at that stage.

Up to the phase of the mother star the karyokinetic figures are of three dimensions, and the diagrams represent optical sections; but in the next stage the loops begin to be compressed towards the equatorial plane, so-called from its relation to the nuclear spindle when this is present (fig. 6), as in vegetable cells and segmenting ova of Echinodermata. This movement Fleming has observed to be again reversed, so that the figure recovers its previous form (fig. 7). He calls the two stages of this movement "the systole and diastole of the star," and illustrates its character by diagrams which are copied in figs. 34 and 35; the phenomenon does not seem to have been noticed by other observers. Ultimately, the loops form the characteristic "equatorial plate" (fig. 8), in which the bends of the loops are directed away from the equatorial plane, their limbs towards it. As the loops form a broad ring, the space within which is empty, and as the bends converge somewhat, they form on each side of the

<sup>1</sup> "Beiträge," ii Theil, 'Archiv Mik. Anat.,' Bd. xviii, 1880.

equatorial plane a figure which may be compared to a shallow basket, or half-opened daisy, with its opening opposed to that of its fellow. These "baskets" now begin to recede from one another, revealing often in the interval between them delicate faint striae which represent the achromatic "nuclear spindle" so conspicuous in the dividing nuclei of the endosperm in Phanerogamia (*vide* Strasburger, 'Zellbildung u. Zelltheilung,' 1880, Taf. i). When they are separated for a certain distance the baskets open out into stars forming the "dyaster" (fig. 10). The cell meanwhile begins to show an equatorial furrow, by the deepening of which it is divided into two daughter cells, each containing a daughter nucleus. The daughter "star" becomes converted into a "wreath" by the junction of the ends of the loops to one another; the wreath passes into a "convolution," and this into a "reticulum," while a membrane appears round the daughter nucleus, nucleoli are formed, and the ground substance again becomes affected by dyes. This inverse metamorphosis is shown in figs. 11—15.

From the part played by nucleoli in this series of changes Flemming concludes that they are merely accumulations of chromatin to form a reserve, which is drawn upon for the formation of fibrils at the commencement of karyokinesis. This view, as he points out, is opposed to all attempts to show a connection between nucleoli and the ultimate branches of nerves.

**Relation of Nuclear Network to Cell-body.**—The intimate structure of the nucleated cell in its resting state is a matter on which observers are still at variance. Klein holds that the cell is composed, like the nucleus, of a reticulum and an intermediate substance, or ground substance, which fills up the meshes of the reticulum; and that the trabeculae of the nuclear reticulum are directly continuous with those belonging to the cell. Flemming, in his latest work, has made some extremely interesting observations on the structure of the resting nucleus. By the help of Seibert's homogeneous immersion and Abbe's illuminating apparatus he has discovered a definite structure in the intermediate substance, which had previously appeared smoothly stained or finely granular. He found in it a much finer network of stained fibrils in connection with the coarse, well-stained trabeculae already known; the fine granulation shown in the ground substance by weaker lenses is the optical expression of the fine network when not resolved. He thinks it probable, though he was unable to determine the fact absolutely, that the substance occupying the meshes of the finer network

was unstained and consisted of achromatin; the finer network is not to be seen in living cells. Flemming studied it in well-stained preparations from the Salamander. He also discovered that the "nuclear membrane" was composed of minute flat plates of chromatic substance in connection with the fibrils of the chromatic network; these plates were separated from each other by slight intervals, so that the membrane seemed to be pierced with holes like a sieve, but he was unable to decide whether the intervals between the plates were really apertures or occupied by achromatic substance. None of Flemming's new observations give any support to Klein's statement that the intranuclear and extranuclear networks are continuous one with the other.

**Finer Structure of the Nuclear Network.**—In a paper which has recently appeared, Dr. W. Pfitzner (ref. 'Morph. Jahrbuch,' vol. vii) of the Anatomical Institute at Heidelberg, states definitely what is brought forward as an opinion by Flemming, viz.: that the chromatin and achromatin are distinct in the resting nucleus, but he makes no mention of a finer as contrasted with a coarser network. The preparations in which he determined this point were sections of the epidermis of the larva of *Salamandra* treated with a 1 per cent. solution of gold-chloride, either examined without further treatment or after exposure to light in a 5 per cent. solution of formic acid. In sections thus prepared the meshes of the network were not stained. Dr. Pfitzner also declares that the nucleoli in the resting nucleus are never connected with the network, but lie free in its meshes, and that there is no "nuclear membrane." The appearance which has been interpreted as due to a membrane is caused by the contrast in optical properties between the achromatin and the surrounding cell protoplasm; he argues that if a membrane were present it would appear, on focussing the surface of the nucleus, as a flat expansion, whereas in reality a network is seen in all optical sections. These are points treated incidentally in this paper, the object of which is to make known certain discoveries in the structure of the chromatic fibrils made by means of a  $\frac{1}{1\frac{1}{2}}$  homogeneous immersion system by Seibert and Kraft. Certain of these fibrils appeared to be composed of a series of granules; and Dr. Pfitzner has come to the conclusion that the chromatic fibril is not homogeneous in structure, but always consists of a moniliform succession of minute spherules of chromatin, held together by an unstained substance which is probably achromatin. He first observed fibrils of this structure in ordinary preparations of the epi-



dermis, glands, branchial epithelium, muscle, cartilage, &c., of the Salamander larva, or adult, stained with hæmatoxylin or saffranin, fixed with chromic or picric acid. The fibrils exhibiting the phenomenon were not common in such specimens, but appeared the more commonly the better the conditions for studying the individual fibrils. It is necessary to have division figures of large size and clearly stained, and the fibrils must be as free as possible from one another. Dr. Pfitzner believes that the granular structure is normal, and that it is generally obscured by physical causes during ordinary processes of preparation; he found that most methods of embedding altered the structure of the fibril; it was best preserved in sections of the epidermis, procured by fastening pieces of the fresh skin of the larva, by means of elder pith, in a microtome. Such sections were first treated with 1 per cent. gold-chloride solution, and then either exposed to light in 5 per cent. formic acid solution, or stained with hæmatoxylin or saffranin, or first exposed to light in the formic acid and then stained. Some fibrils he discovered to consist of a double row of spherules; this state corresponds with the longitudinal splitting observed by Flemming.

A large portion of the paper is devoted to the support of the startling proposition that these chromatin spherules are individual molecules in the chemical and physical sense of the word. This idea is based on the argument that the more highly differentiated a living substance is the higher is its molecular weight, the larger the number of atoms the molecule contains, and the greater its size. Proceeding on an assumption so slenderly supported, Dr. Pfitzner attempts to develop a theory of the whole process of life and division in the cell, but his pages are scarcely less difficult to comprehend than the phenomena they are intended to explain.

Klein's view that the intranuclear network is continuous with that of the cell is supported by Prof. C. Frommann, of Jena ('*Jenaische Zeitschr.*,' Bd. 14, 1880), who has convinced himself that there is no membranous envelope enclosing either the nucleus or the cell itself. The contours seen round the nucleus and the cell are due to the presence of superficial fibrils (*Grenzfäden*), which run for a longer or shorter distance along the surface, and are connected by other fibrils with the contiguous networks.

Klein studied indirect division in the epithelial cells of the bladder of the newt, in the lower layers of the epidermis of the sheep, and other structures. According to his results, the star- and wreath-form of the mother nucleus are of only



two dimensions, not of three, as in Flemming's description; he considers them to occur after the mass of fibrils has been compressed into the equatorial plane. He states, too, that the central points of the daughter nuclei may separate before the fibrils have divided into loops, a division of the fibrils in the equatorial plane occurring subsequently. Flemming has observed in the cells of the testes of Salamander "cask forms," in which the fibrils were continuous across the equator; but he believes this to be the consequence of temporary fusion of the ends of fibrils previously separate; for in earlier stages there were distinct loops only half as long as the cask figure.

**Nuclear Spindle.**—The nuclear spindle, first described by Bütschli,<sup>1</sup> is most conspicuous in the dividing cells of plants and segmenting ova; it is formed of faint striæ passing between two poles, situated at equal distances from the equatorial plate; frequently at each pole there is a radiating figure or "sun," composed of faint striæ, like those of the spindle. The spindle and suns are well shown in figs. 16—19 from the segmenting ovum of *Toxopneustes lividus*. Flemming considers the spindle to consist of achromatin, and therefore to belong to the nucleus, while Strasburger,<sup>2</sup> with whom Klein agrees, believes that it belongs to the cell protoplasm. It is more probable that the suns at the poles of the spindle are parts of the cell substance. This is proved for the ova of Echinoderms by Flemming's figures in the third part of his 'Beiträge.' Strasburger gives a typical figure of a dividing nucleus with polar suns, spindle, and equatorial plate, from the embryo sac of *Viola palustris* (ref. op. cit., Taf. ii, fig. 33).

The descriptions of other observers do not resemble those of Flemming in giving such definite forms to the chromatic constituents of the nucleus. Schleicher<sup>1</sup> represents the chromatic elements in the cartilage cells of Batrachian larvæ as irregular granules and rods, with a general tendency to lie parallel to the axis of the spindle. These, when they begin to form the daughter nuclei, fuse together into clumps of chromatic substance, which subsequently again break up into rods and granules. Figs. 27, 28, and 29, which are successive stages, illustrate this; figs. 30 and 31, from the cranial cartilage of a toad, show the achromatic striæ more clearly.

**Definite Number of the Chromatic Loops.**—In three cases of

<sup>1</sup> 'Zeitschr. Wiss. Zool.,' vol. 25, 1875.

<sup>2</sup> 'Zellbildung u. Zelltheilung,' 1880, p. 328.

cells from the epithelium of the mouth and branchiæ of the larva of *Salamandra*, Flemming, in his most recent researches, was able to count the number of chromatic loops present. The number in each case was twenty-four, and in other cases, where the number could not be determined, twenty-four was the probable total. He has also seen under Seibert's oil immersion a highly refractive particle at the poles of the achromatic spindle, towards which the threads of the spindle converge. This body has the same relations as the polar corpuscle which Fol has described in egg cells. In some preparations the chromatic loops were seen to lie each with its bend on one of the achromatic threads (figs. 41, 42, and 43).

**Differences between Strasburger and Flemming.**—In the figures of vegetable cells given by Strasburger, rods and granules almost universally take the place of the regular looped threads described by Flemming. He differs, also, in many other points from Flemming, who devotes a great part of his latest paper to the discussion of Strasburger's views. If Strasburger's observations are conclusive it follows that the karyokinesis of vegetable cells is a process agreeing only in the most general features with that which Flemming has seen in *Salamandra*, and that there is no exact correspondence between the successive stages in the two cases. The only stage where any close resemblance exists is that of the equatorial plate, where the achromatic spindle, with the disc of chromatic elements at its equator, is obviously homologous with the corresponding stage described by Flemming in *Amphibia*, and Fol and others in segmenting ova. (For a complete series of the successive changes, according to Strasburger, see figs. 85—108, Taf. 3 and 4, 'Zellbildung u. Zelltheilung,' 1880, from *Lilium martagon*; and figs. 60—69, Taf. 8, from *Iris pumilla*.) The resting nucleus is figured by Strasburger as consisting of large round granules of chromatic substance irregularly distributed in a homogeneous unstained matrix. Out of these granules is formed a structure having a distant resemblance to a convolution which does not break up into regular loops, and forms no mother star, either of three dimensions or of flattened form, but by irregular changes assumes a spindle form, which, in the embryo sac of *Lilium martagon*, consists of a continuous mass of chromatic substance at the equator, with threads of different lengths and thicknesses passing from it towards the poles. A division of the chromatin takes place at the equator and the threads travel towards

<sup>1</sup> 'Archiv. Mik. Anat.,' Bd. 16,

the poles, forming a dyaster<sup>1</sup>, the striæ of the achromatic spindle at the same time becoming visible. The chromatic threads at each pole then fuse together to form the daughter nucleus, which ultimately becomes granular, like the resting nucleus from which the process commenced. The two chief points in which this description of the process differs from Flemming's are—1st. The division at the equator of chromatic substance previously continuous; and 2nd. The fusion of threads to form the daughter nucleus.

On the first of these points Strasburger is so satisfied that he draws the general conclusion (op. cit., p. 331) that whatever chromatic element lies in the equatorial plane, or crosses it at the time of division, is divided in the position it happens to have assumed. If there are any equatorially placed threads they are divided longitudinally. The second point is in direct opposition to Flemming's conclusion that the daughter nucleus, in its return to the resting state, passes through the same phases in reversed order as the mother nucleus before division. Flemming has applied to Strasburger's work a crucial test, the result of which showed that the peculiarities in the observations of the latter were due to imperfections in his methods of preparation and examination. Preparations of the protoplasm of the embryo sac of *Lilium croceum* were sent to Flemming by Herr Soltwedel, whose preparations were in part the material for Strasburger's work. These had been fixed with alcohol, stained with borax-carmin and methyl-green, and mounted in glycerine. Their appearance under the microscope corresponded exactly with Strasburger's figures. Flemming took off the cover-glass from three of these, restained them with alum-carmin, cleared them with oil of cloves, and mounted them in dammar varnish. He then examined them with Seibert's homogeneous immersion  $\frac{1}{1\frac{1}{2}}$ th, and Abbe's illuminating apparatus, and found that the structure of the nuclear figures was the same as in those from Salamandra. There was no chromatic substance continuous across the equatorial plane, but an equatorial plate formed of two

<sup>1</sup> This term I take from Klein in his 'Atlas of Histology,' 1880. Flemming usually distinguishes the same stage as that of the "Tochter-sterne." It is not to be confused with the nuclear spindle having a polar sun at each end, which, in the ovum of Asterias and other Echinoderms, precedes the formation of a directive vesicle, and which occurs again in the segmentation of the ovum. This appearance, which Auerbach called the "karyolitic figure," corresponds to Flemming's phase showing an equatorial plate. The earlier views on the subject of cell division were put before readers of this Journal in the number for April, 1876, by Mr. Priestley, of Manchester.

collections of regular loops with their free ends turned towards the plane; and there was no fusion of the loops after their removal to the poles; they formed the wreath and convolution as in typical cases.

In the formation of endosperm in the embryo sac of *Phanerogamia*, the division of the nucleus is not immediately followed by that of the cell, but after the full number of potential cells has been formed, cell walls arise in a peculiar way. Each pair of nuclei is connected by the striæ of the achromatic spindle, and at the equator of this there appears a series of thickenings, which Strasburger believes, from their behaviour to iodine, to consist of starch or some allied substance; out of these is formed the partition wall of cellulose between the newly formed cells. Strasburger names this series of thickenings the "cell plate," a term which Klein has used to denote the body of the cell as distinguished from its nucleus. In the dividing testicular cells of *Salamandra*, in which the achromatic spindle is very conspicuous, Flemming has seen equatorial thickenings corresponding to the cell plate of plants; but as neither these nor the spindle were to be seen in the living cells, he was unable to determine their function. He also found a corresponding structure at the equator of the achromatic spindle of the first dividing nucleus in *Echinoderm* ova ('*Beiträge*,' iii Theil); this was figured by Fol<sup>1</sup> under the name "filaments connectifs." The appearance seemed to Flemming to be caused by a bending and winding of each fibril of the spindle for a short distance, and not to be due to swellings or thickenings.

**Development of Spermatozoa.**—Flemming succeeded in following the nuclear changes in actual progress in living cells from the testes of *Salamandra*; the fibrils when first observed were already in the form of numerous loops, these passed towards the poles of the nuclear space and back towards the equator in alternate systole and diastole before the formation of the equatorial plate. The following stages took place in accordance with his general description, except that no longitudinal splitting was observed; of this phenomenon he was unable to obtain evidence in *Triton*, *Batrachia*, plants, and mammals. Pere-meschko<sup>2</sup> has observed the division of living cells *in situ* in the larva of *Triton cristatus*. Epithelium cells, stellate connective-tissue cells, white blood-corpuscles, and the spindle-shaped cells of vasifactive tissue, showed the same

<sup>1</sup> '*Fécondation et commencement de l'hénogenie*,' Geneva, 1879.

<sup>2</sup> '*Archiv Mik. Anat.*,' Bd. 16.



series of changes. The resting nucleus enclosed by a membrane was invisible, but nuclei about to divide contained granules and threads which assumed the characteristic forms.

**Division of Nuclei into more than two parts.**—Several observers have described cases of the karyokinetic division of nuclei into more than two parts. Eberth<sup>1</sup> studied indirect division in tissues which were in process of regeneration after artificial injury. He either cut with a scalpel or destroyed with chloride of zinc portions of the epithelium of the cornea, and of Descemet's membrane in the rabbit and the frog, then, after some days, made chloride of gold preparations from the injured parts. He believed that some of the appearances he saw were due to the simultaneous division of a nucleus into several parts. The four young nuclei, which he figures lying close together and apparently of the same age, may have all proceeded from a single mother nucleus, but there is little reason to suppose that the nucleus he describes with seven pointed processes (fig. 32) was preparing to divide into seven parts.

More detailed evidence has been brought forward by Julius Arnold,<sup>2</sup> whose preparations were from examples of epithelioma, carcinoma, and sarcoma of the human subject. He describes nuclei with three and four processes (figs. 20 and 21) which have a distinct membrane, the interior being filled with short rods thickened at one end. The character of these is very abnormal. More convincing are other figures he gives of spherical nuclei with a distinct membrane, and containing a triradial arrangement of granules in double rows; from the granules pale striæ pass towards three foci or poles (figs. 25 and 26).

Dr. Louis Waldstein was kind enough, at the request of Prof. Laukester, to show me his preparations from tumours of the same kind as those which Arnold used. In these triradial figures were to be found similar to those described by Arnold. Their appearance is shown in fig. 39. The rays of the figure did not consist of double rows of granules, but indications of fine fibrils proceeding towards three poles were visible. I saw no membrane around nuclei in this state. From the appearance of these triradial forms it seems probable that in the figures of Arnold, which I have copied in figs. 20 and 21, only the chromatic part of the nucleus is represented corresponding to the granules in figs. 25 and 26.

<sup>1</sup> 'Virchow's Archiv,' Bd. 67, 1876.

<sup>2</sup> Ibid., Bd. 78, 1879.

Dr. Waldstein has found both ordinary and triradiate karyokinetic figures in a tissue where they have not hitherto been discovered, viz. in the marrow of human bone. The marrow in question came from a man who died of leucocythæmia, and was much hypertrophied, as also were the spleen, liver, and lymphatic glands. Dr. Waldstein intends to publish shortly his observations on this case.

Strasburger found indications of the division of one nucleus into three in the endosperm of *Reseda odorata*. In all these cases cell multiplication is taking place very rapidly; in animals no instance is recorded of karyokinetic figures with more than two poles in healthy tissues, they occur only where the rate of growth is abnormally high.<sup>1</sup>

**Polar Cells or Directive Corpuseles of the Ovum.**—The formation of polar cells in many ova, like the segmentation of the ovum after fertilization, has been recently discovered to depend on a process of karyokinesis. The germinal vesicle, which was previously believed to degenerate, was seen by Fol and Hertwig in the ovum of *Asterias glacialis* to pass into the spindle form, and one end of the spindle was traced into the polar cell.<sup>2</sup> Since this process is, as Hertwig has pointed out, essentially a cell division by the indirect method, it may be homologous with the division

<sup>1</sup> Since the above was written, new observations on the multiple division of nuclei have been published in 'Virchow's Archiv,' Bd. 86, by W. A. Martin. The observations were made at the Pathological-anatomical Institute of Heidelberg University, and were confined to preparations from a single cancer of the mammary gland, which had developed with extreme rapidity. Two of the most striking figures given by Mr. Martin are copied in Figs. 45 and 46 of the Plate illustrating this review. Mr. Martin figures the nuclear plate in the same way as Arnold, *i.e.* as double rows of granules; but he also figures and describes typical convolutions between the limbs of the nuclear plate. It is difficult to reconcile the coexistence of nuclear plate and convolutions with the conception of karyokinesis formed by other observers, especially that of Flemming. Mr. Martin makes no attempt to lessen the surprise caused by his description; he seems to regard the convolutions as formed of the same substance as the half spindles, which occupy some of the spaces between the rays of the nuclear plate. This would be the first observation of achromatic fibrils in the form of the convolution. It is to be noted that Mr. Martin's material was hardened only by spirit. It is much to be desired that some pathologist should consider the cell division in tumours from the same point of view as Flemming and others, who wish to discover a universal type of karyokinesis. Mr. Martin has advanced the knowledge of multiple division in tumours a step further, and has proved that a cell may give rise to four and even seven daughter cells simultaneously by karyokinetic changes; but more work upon this interesting subject will be very welcome.

<sup>2</sup> Balfour, 'Comp. Embryology,' Pt. 1. Also 'On the Phenomena accompanying the Maturation and Impregnation of the Ovum,' this Journal, vol. xviii, 1878.

of the spermatospore to form several spermatozoa. The difference between the two processes could be explained by the fact that the ovum requires for its development a large supply of food material. One of the daughter ova has been selected to develop, and possesses all the food material, while the others remain small and incapable of forming an embryo. On the other hand, fertilization is more certainly ensured by a number of small spermatozoa than by a few of larger size, and thus the division of the mother cell of the *male* element is a process of functional importance, while in the female it has become rudimentary.

**Do Cells Divide without Karyokinesis?**—Since the process of karyokinesis occurs in such diverse parts of both the animal and vegetable kingdoms, there is ground for inquiring whether it is universal. Flemming doubts whether cell multiplication by any other method has yet been definitely proved. Klein, in a recent number of this Journal (July, 1879), attempted to prove from the observed ratios of karyokinetic figures to resting nuclei in preparations from the epithelium of the newt, that the number of indirect divisions was insufficient to account for the rapid regeneration of that epithelium. To this Flemming replies by calculating from the same data and proving that the ratios are sufficient. Van Beneden believed that multinuclear cells arise by direct division of the nucleus of a uninucleate cell, but the indirect division of a nucleus has been often observed to take place without any division of the cell containing it. Multinuclear cells are frequently found with all their nuclei in process of indirect division; they are generally all in the same phase though not invariably. The division of *Amœbæ* and amœboid cells seems to be unaccompanied by karyokinetic changes, but the nuclei of these are very small and difficult to observe. F. Schmitz<sup>1</sup> has described a direct division in vegetable cells without karyokinetic changes.

Flemming found karyokinetic figures in colourless cells in the blood of a man suffering from leucocythæmia, but these were not sufficiently common to account for the whole number of such cells, and he thinks it doubtful whether the numerous colourless cells of the blood in leucocythæmia are the same as the colourless blood cells of normal blood; they are more probably young stages of red cells derived from the spleen and bone-marrow. On the other hand, Franz Schultze<sup>2</sup> was fortunate enough to observe a living specimen of *Amœba*

<sup>1</sup> S. B. Niederrhein, Ges. Naturwiss. u. Heilkun. Bonn, 1880.

<sup>2</sup> 'Archiv, 'Mik. Anat.,' Bd. 11, 1875.

*polypodia* in the process of division, and the changes which, according to his description, took place in the nucleus, were very different to those of karyokinesis. The nucleus, which was large, single, and well defined, first became dumb-bell-shaped, and then divided by an increased tension of the central part. Other infusoria, which have been observed, conform in dividing more or less closely to the karyokinetic laws. The dividing nucleus of the flagellate *Anisonema sulcatum*, figured by Bütschli,<sup>1</sup> is not unlike an achromatic spindle with a chromatic figure at each end. The large nucleus of the peritrichous species *Spirochona gemmipara*, according to R. Hertwig,<sup>2</sup> is longitudinally striated during division, though it differs widely from a typical nuclear spindle. The shape of the nucleus of the acinetan *Podophrya quadripartita*, figured by Bütschli,<sup>3</sup> is unlike anything found in karyokinesis; but here also the cellular bud encloses a segment of the nucleus.

Dr. Gruber, in his recent memoirs on the process of division in monothalamous Rhizopoda ('Zeitschr. wiss. Zool.,' Bds. 35 and 36), has discussed the relation of the peculiar phenomena discovered by him to indirect cell-division. He shows how the rapid formation of a new animal at the mouth of the mother, out of protoplasm and shell-plates accumulated for the purpose beforehand, is easily derived from a division like that of *Amœba*. The presence of a firm shell prevents the actual division of the animal, while by the previous formation of shell-plates in the maternal protoplasm the new individual is provided with a protective covering at the commencement of its separate existence. The division of the nucleus does not take place till the new animal is already complete, and this Dr. Gruber considers supports Strasburger's view that the division of the cell is independent of that of the nucleus; and it also shows that the whole process proceeds from the activity of the cell-protoplasm, the nucleus playing a subordinate part. In *Euglypha alveolata* the nucleus, before dividing, either became granular or "gewundene Linien" appeared in it, then it elongated and showed longitudinal lines. Dr. Gruber thinks that these changes represent Flemming's convolution and equatorial plate. If so, the resemblance between the indirect division in Rhizopods and the cells of *Salamandra* is not very close.

Free Formation of Nuclei.—Another question which pre-

<sup>1</sup> 'Zeitschr. Wiss. Zool.,' Bd. 30, fig. 18c.

<sup>2</sup> 'Jenaische Zeitschr.,' vol. xi.

<sup>3</sup> *Ib.*, vol. x.



sents itself in connection with this subject is, whether there is any free formation of nuclei independent of pre-existing nuclei. Such a free formation would seem to take place, according to the researches of Van Beneden, in the endodermal cell of Dicyemidæ. F. Schmitz (loc. cit.) was unable to find nuclei in the cells of Phycochromaceæ, so that these at present form an exception to the general rule, that when a cell divides each portion includes a portion of the original nucleus. But it is not long since the independent origin of nuclei in the ovum of animals and the embryo-sac of Phanerogamia was generally accepted; and it is probable that future researches will enable the questions still unsettled, with reference to the multiplication of cells in cases which now seem exceptional, to be more definitely decided.

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NOTE on the ORGAN of JACOBSON. By REUBEN T. HARVEY, M.D., F.R.C.P., Lecturer on Physiology in the Carmichael Medical School, Dublin.

DURING the spring of this year, when engaged in some work on the histology of the nasal mucous membrane in the mouse, my attention was directed by Dr. Klein's paper in the January number of this Journal to the organ of Jacobson. The distinctly olfactory character of the epithelium in this organ induced me to seek for the junction which was supposed to exist between it and the mouth, with a view of determining how the transition in the character of the epithelium took place, or of discovering some vestige of the connection which exists in the early foetal condition between these tubes and the nasal cavity. With the the material which I then had at my disposal, I failed to discover any communication between the organ of Jacobson and the mouth; and my sections left no doubt on my mind but that the opening from it was into the nostril, although they did not demonstrate the exact manner in which such opening took place.

For want of time and suitable material I was obliged to postpone my investigations until the conclusion of the winter session. On resuming, I had no difficulty in obtaining a series of vertical sections from before backwards of a mouse's head, which demonstrated the opening sought for. Four days after this I received the April number of the Journal containing Dr. Klein's second paper, in which he records a similar discovery in the guinea-pig. I have since worked out the point in the kitten and hedgehog, and I desire now briefly to describe some of the more important facts in each case.

I. *The Mouse*.—The manner in which Jacobson's organ is connected with the nostril in the mouse is very similar to what Klein describes in the guinea-pig. In successive sections from before backwards the following stages may be observed:—First, the mucous membrane lining the chink, into which the floor of the nostril is narrowed in front, is composed of a stratified scaly epithelium, which stains much more intensely than the rest of the nasal epithelium. Secondly, the inferior margin of this chink bends inwards and upwards into the septum under a small isolated mass of so-called cellular cartilage. Thirdly, as this depression into the septum deepens it becomes somewhat flask-shaped, its orifice becoming elongated and narrowed. Ultimately the neck becomes obliterated, and we have then to deal with the section of a complete tube. Lastly, the layer of scaly epithelium is replaced by columnar, and the inner surface of the

tubes becomes much thickened, assuming all the characters of olfactory epithelium. The outer layer of epithelium bends in towards the lumen of the tube, making the section of the organ assume a reniform appearance, in the hilus of which may be seen a large nerve embedded in glands. At this stage the mass of cartilage is no longer visible.

The organ of Jacobson, then, in the mouse has no connection with the Stenonian canal. It is simply a tubular recess from the nostril, situated in the septum. There is, moreover, no surrounding cartilage.

In the cat and hedgehog the case is quite different. In these animals there is a direct communication between the organ in question at its anterior extremity and the Stenonian canal, where this latter tube enters the nostril.

II. *The Cat*.—My observations were made on the heads of three kittens, taken from the uterus of a cat apparently about three days before full time.

In a vertical section at the spot where the Stenonian canal enters the mouth the thickness of the bone (containing the lateral incisor teeth) is considerable. The epithelium on the floor of the nostril is of the compound scaly variety, as in the mouse. The canal in this position is seen passing upwards and outwards, being overhung by a horseshoe of hyaline cartilage. In a section a little further back, the canal is seen bending vertically upwards, and the cartilage becomes situated on its inner side. In its passage upwards to the nostril the canal passes but very slightly backwards, and hence vertical sections cut it in considerable length. From the inner side of such a section of the canal a diverticulum is seen passing inwards, which is received into a concavity in the cartilage. This diverticulum is the commencement of the organ of Jacobson. The Stenonian canal opens into the anterior angle of a deep vertical chink, into which the floor of the nostril is narrowed in front. The anterior boundary of this chink is almost a vertical line, and is formed by the thick mass of bone containing the incisor teeth. In a section where the canal enters the nostril this might be taken for a continuation of the canal. But that such is not the case is proved in the section itself by the fact that the outer surface of the chink is covered by cylindrical epithelium, and in subsequent sections by their showing the change in level that has taken place in the upper surface of the palate. The section of the organ of Jacobson is now seen in the side of the septum, and this change has taken place without its distance from the under surface of the palate having become materially changed, and hence it is caused by the sudden deepening of the nostril, and not by any marked upward direction of its own tube. At ten

actual spot where the Stenonian canal opens into this chink the organ of Jacobson is still an unclosed diverticulum. The cartilage has, however, become C-shaped, and the neck of the diverticulum constricted; and a very little farther back the neck has become obliterated, and the cartilage is a complete ring. The epithelium of the tube now assumes the cylindrical form, and in the connective tissue which separates it from the cartilage numerous Bowman's glands are embedded.

III. *The Hedgehog*.—My observations in the case of the hedgehog are confined to a single specimen—an old male—just coming out of the state of hibernation.

The state of things found in this animal is, in the main, similar to that found in the kitten. The Stenonian canals open into the mouth at either side of a papilla. As we trace them to the nose, we find them running a more sinuous course than in the kitten. At first they pass upwards, and outwards, and have a rim of cartilage on their outer sides. They then assume the general direction of upwards and backwards, and in successive sections the cartilage is found first at the outer side, then above, and lastly to the inner side. When this last stage is reached the commencement of the organ of Jacobson is seen, just, as in the cat, as a diverticulum, which is received into the concavity of the cartilage. At the spot where the Stenonian canal enters the nostril there is free lateral communication between Jacobson's organ and the nostril, but immediately behind this point both cartilage and epithelium undergo much the same modifications as described in the case of the cat. The deepening of the floor of the nostril is comparatively gradual, not sudden—as in the cat—and the two tubes which run side by side, with very much the same direction, enter it obliquely. In the cat the tubes are nearly at right angles, the organ of Jacobson being nearly horizontal, and the Stenonian canal nearly vertical. There is an appearance on the surface of the crest of bone into which the cartilage of the nasal septum is inserted which is extremely like the commencement of the organ of Jacobson in the mouse, namely, an overhanging ledge. But on tracing this backwards we find it still persistent at the spot where the organ enters the nostril, and, though immediately above it, still quite distinct from it.

In the present paper I have only described what may be called the rough anatomical relations of the organ of Jacobson in the three animals, as I think they may be of interest in connection with Dr. Klein's papers. I hope subsequently to record my observations, as yet incomplete, of the minute structure of the nasal epithelium.



ON DREPANIDIUM RANARUM, *the CELL-PARASITE of the FROG'S BLOOD and SPLEEN* (Gaule's Würmschen). By E. RAY LANKESTER, M.A., F.R.S., Jodrell Professor of Zoology in University College, London.

IN the year 1871 I described and figured in this Journal (vol. xi, p. 389), certain minute sausage-like parasites which I had found in the blood of *Rana esculenta*. I showed that these parasites were sometimes to be met with attached by one end to a red blood-corpuscle, and I exhibited in my drawing of them (here reproduced, figs. 3 and 4), the sharply pointed character of one end of the cylindrical body, the differentiation of their substance into bands of greater and less refringency, and the definite curvature of the sausage-like form which they present. I suggested that these parasites were possibly connected with the life-history of the *Trypanosoma sanguinis* of Gruby, which I had observed and supposed to be an undescribed organism. At the same time I pointed out their resemblance to certain peculiar spores which I had observed in the cysts of a Gregarina parasitic in Tubifex. These parasites have, after a lapse of ten years, been re-discovered by Dr. Gaule, whilst working in the Physiological Institute of Professor Ludwig at Leipzig, where I had originally observed them. Dr. Gaule has published two lengthy papers upon them in the 'Archiv für Physiologie,' 1880 and 1881. In the first of these papers he gave no figure of the object which he was describing, nor did he refer to the fact that it was already known. This omission, together with the exceedingly original views which Dr. Gaule advanced as to the nature of the parasites, led to a complete misunderstanding of his observations, so that it was supposed that he had been studying one of those curious phenomena of disintegration of the red blood-corpuscle which, from the date of Addison's paper in this Journal (1861) to the present day, have much occupied the attention of histologists. Misled in this manner by the absence of drawings from Dr. Gaule's paper, and by the strange method of reasoning characteristic of that observer, Mr. Dowdeswell reported upon these so-called "Würmschen" in this Journal, January, 1881, p. 160, and referred the bodies described by Gaule to the category of disintegration products.

It is now, however, evident from Dr. Gaule's second paper, which is accompanied by a plate, that his "Würmschen" are the parasites described by me, a fact which he has become aware of himself.

It is well that a definite systematic name should be employed for these parasites, if only to avoid the misleading name of "Würmschen" applied to them by Dr. Gaule. The term "Cytzoa," of which this writer also makes use, is not applic-

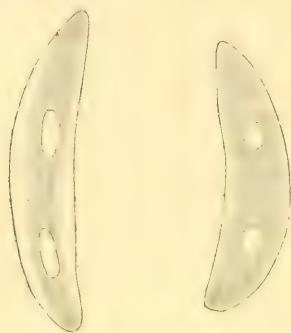


FIG. 1.—Two specimens of *Drepanidium ranarum*, treated with iodine and showing refringent granules.

able to these parasites in a generic sense, since we are acquainted with other cell-parasites which illustrate and explain the curious

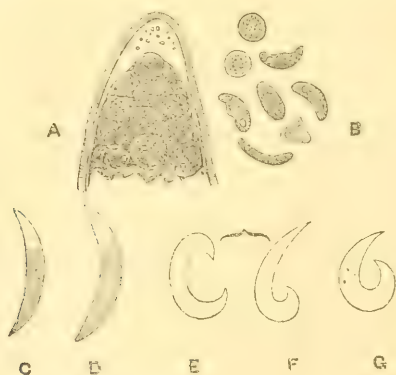


FIG. 2.—A. One end of the sac-like parasite of mammalian muscular fibre, here designated *Sarcocystis Miescheri* (after Leuckart). B. *Drepanidium*-form of young liberated from spore-like bodies in the same. C D. *Drepanidium*-form of young from cysts of *Coccidium* of the house mouse. E F G. The same in movement (after Eimer).

relationship between this parasite and the red blood corpuscle, and the word Cytzoa may therefore be better used as a designa-

tion descriptive of cell-parasites generally. I propose to enter the parasite of the cells of the frog's blood and spleen upon the list of recognised organisms as *Drepanidium ranarum*.

Dr. Gaule has added two interesting facts to those which I was able to furnish in 1871, relative to *Drepanidium*. The more important is that not only is *Drepanidium* to be found attached to the red blood-corpuscle as I had figured it, but that it occurs also *within* the substance of the corpuscle. In the cells of the spleen these parasites are also shown by Dr. Gaule to occur in some abundance, and the spleen furnishes the

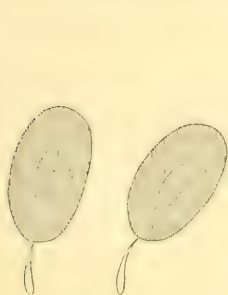


FIG. 3.



FIG. 4.

FIG. 3.—*Drepanidium ranarum* attached to red blood-corpuscles of *Rana esculenta*. (Original figure, 1871.)

FIG. 4.—Two *Drepanidia ranarum* more highly magnified. One shows two motionless filaments. (Original figure, 1871.)

most ready source for procuring them for observation. They are admitted by Dr. Gaule to occur in the free condition floating in the plasma of the blood, both in the spleen and in other parts of the body.<sup>1</sup>

The second fact of interest for which we are indebted to Dr. Gaule, is that when a blood-preparation (with cover-glass closed by a film of oil) containing these parasites is heated on the stage of the microscope to 30—35° C. the parasites exhibit very active and characteristic movements.

I am indebted to Dr. Woolridge, of Guy's Hospital, for the opportunity of again studying the *Drepanidium ranarum*, he having kindly placed at my disposal two specimens of the *Rana esculenta* which were presented to him by Dr. Gaule, and had been ascertained to be infested with these parasites. The frogs were examined in the month of November, and contained a number of the parasites, but not so large a number as appear to have been present in some cases studied by Dr. Gaule. I found the teasing of a small piece of spleen in salt-solution to

<sup>1</sup> I learn from Dr. Woolridge that Dr. Gaule has found the *Drepanidia* also in the liver and pancreas of the Frog.

be the readiest means of obtaining a preparation showing specimens of the *Drepanidium*, though a few were also observed in the blood.

I am able to confirm completely Dr. Gaule's account of the remarkable movements exhibited by the parasites when the preparation containing them is warmed to about  $35^{\circ}$  C. on the stage of the microscope. These movements would indeed be very extraordinary were we dealing with anything but an independent protoplasmic organism. To the zoologist, accustomed to the observation of the simpler forms of life, they present nothing paradoxical. Probably under certain circumstances which recur in the natural state these parasites exhibit the active movements which I have only, as yet, succeeded in witnessing when the temperature is artificially raised to that point which is known to be most favorable in general to the activity of contractile protoplasm. The movements are caused by an alternate bending and straightening of the curved and spirally twisted body of the *Drepanidium*. They are certainly *not* caused by any attached filament of protoplasm in the form of a flagellum (either "tractellum" or "pulscellum"), though, as seen in my original figure here reproduced (fig. 4), and in the specimens examined this year by me, occasionally a very fine motionless filament, or even two, may be attached to one end of the *Drepanidium*.

The movements of the *Drepanidium* cease altogether when the preparation containing it is heated to a temperature of  $70^{\circ}$  C., a fact tending very strongly to establish (what there is no reason of another kind for doubting) that the movement is a "vital" one, that is to say, depends on the substance of the *Drepanidium* being in that physical condition which we call "living," and which, in most cases, is irrevocably disturbed by exposure to a temperature of  $70^{\circ}$  C.

Like many other minute organisms (*e.g.* certain Bacteria), *Drepanidium* is probably motionless for long periods under normal circumstances, and has an active period which may be induced by a favourable rise of temperature.

*Affinities of Drepanidium.*—Since my first notice of *Drepanidium* our knowledge of the Gregarinidæ and their allies, or as Rudolph Leuckart has very aptly termed them, the "Sporozoa," has been greatly extended, chiefly through the publications of Eimer ('*Psorospermien*,' Wurzburg, 1870), Aimée Schneider ('*Archives de Zoologie experimentale*,' vol. iv, p. 493, 1875), and of Butschli ('*Zeitschr. wiss. Zoologie*,' vol. xxxv, pp. 384 and 629, 1881).

It has now in fact become evident that the Sporozoa are at a certain period of their life-history very usually *cell parasites*.



It was inferred from Lieberkühn's descriptions and figures of *Gregarines velues* (noticed also by Stein), that some, at least, of the forms of Gregarina to be met with in the testicular sac of the Earthworm, which were enveloped in a vesicle carrying conical processes on its surface, were in fact sperm polyplasts which had been penetrated in an early condition of their development by young Gregarinæ.<sup>1</sup> The Gregarina had grown and more or less tightly fitted to its cell host, which also had proceeded on its development and produced in a somewhat abnormal form its crop of spermatoblasts.

I do not mean to say that all the varieties of Gregarinæ with peculiar cuticle-like investments, which are to be observed in the Earthworm are to be explained as the result of the parasitism of the Gregarina in a sperm-cell. But such forms as those figured by Lieberkühn in the plate i, fig. 7, of his 'Evolution des Gregarines' (published as a *mémoire couronné* (1854) by the Belgian Academy), and such as figured in my Plate VII, figs. 26, 28 of this Journal, 1863, illustrating a notice of "Our present knowledge of the Gregarinæ," certainly are instances of this mode of parasitism. More recently, Butschli ('Zeitsch. wiss. Zool.,' vol. xxxv, 1881) has shown that sometimes Gregarinæ parasitic in the Earthworm penetrate the epithelial cells of the ciliated funnels of the spermatid duct, and such a Gregarina may remain implanted by one end in the cell after attaining fifty times the linear dimensions of the cell in which it was once parasitic.

The cell-parasitism of the Sporozoa at a certain stage of their existence has been chiefly demonstrated through Eimer's observations on the oviform psorosperms (for which Leuckart adopts the generic name *Coccidium*) of the House-mouse, and through Aimée Schneider's discovery of the falciform corpuscles which form within the pseudonaviculæ of *Monocystis lumbrici* (see fig. 5c) and other Gregarinæ, and serve by their presence to effectually establish the relationship of the several phases in the life-history of Eimer's *Coccidium* with the several phases in the life-history of typical Gregarinæ.

Eimer found spherical Gregarinæ (*Coccidium*) existing as cell-parasites in the epithelial cells of the intestine of the House-mouse.<sup>2</sup> This minute Gregarina, without great increase of size, envelopes itself in a transparent cyst, and breaks up internally

<sup>1</sup> See especially Lieberkühn, in 'Reichert and Du Bois Reymond's Archiv,' 1865.

<sup>2</sup> Lieberkühn also describes and figures the *Coccidium oviforme* of the rabbit as a cell-parasite in the intestinal epithelium in his 'Evolution des Gregarines,' already cited, 1854. A valuable and detailed history of our knowledge of the Sporozoa, especially of Coccidia, is given by Leuckart in the new edition of his 'Parasiten des Menschen,' part i, p. 241, 1879.

into spores (corresponding to the pseudonaviculæ of other Gregarinæ), which in turn break up into bundles of "falciform corpuscles." These falciform corpuscles are set free from the spore-case and from the cyst, and exhibit active contractions (woodcut, fig. 2 c, d, e, f, g). According to Eimer, the falciform corpuscles are capable of assuming an amœboid form, but it is probable that under natural conditions they penetrate the cells of the tissues of the Mouse before assuming a change of form, which brings them to the condition of the spherical Gregarina from which they started.

It is the merit of M. Aimée Schneider to have refuted the observations of Lieberkühn, according to which the "pseudonavicula" or "spore" of the *Monocystis lumbrici* gives exit to an amœboid particle, and to have shown (by the use of the modern method of osmic acid fixing and picro-carminic staining) that, on the contrary, just as in Eimer's Coccidium, each of the spores formed by the encysted Gregarina breaks up into six or a less number of falciform corpuscles very closely similar in form to those observed by Eimer. These falciform corpuscles—the young stage of the *Monocystis lumbrici*—are nucleated and probably pass by direct development into the adult form.

But as we have seen, they often exhibit the habit of cell-parasitism observed in Eimer's Coccidium, and pass when in the falciform phase of growth into the substance of the young sperm cells or epithelial cells of their host.

A. Schneider has described further, and figured the formation of identical falciform corpuscles in the spores (pseudonaviculæ) of *Monocystis terebellæ* and of *M. nemertis*.

I have recently observed a similar formation of falciform young in the naviculoid spores of the *Monocystis* parasitic in *Thalassema Neptuni*, Gaertner—and have moreover found the young *Monocystis* as a *cell-parasite* within the epithelial cells of the intestine, as also in one specimen within the ova in great numbers.

Bütschli (loc. cit.) has recently described important cases of cell-parasitism on the part of the young of *Gregarina blattarum* and of a Gregarina occurring in the intestine of *Lithobius forficatus*. In both cases the young Gregarinæ were observed *within* the cells forming the lining of the alimentary tract. Bütschli has carefully figured the spores of *G. blattarum*, and it seems doubtful whether such exceedingly minute spores as these give rise to more than one "falciform embryo." On the other hand, in the case of *Lithobius forficatus* he observed groups of falciform bodies comparable to those formed in a spore or pseudonavicula of a *Monocystis*. It is probable that they were not related to the large Gregarina of *Lithobius*, but to the *Monocystid* *Adelia ovata* discovered in that animal by A. Schneider.

From these facts it is sufficiently evident that the Sporozoa are eminently *cell*-parasites, that the falciform motile young which escape from their spores penetrate the cells of the hosts in which these animals are parasitic, and that whilst the smaller forms (Coccidia) often attain the reproductive phase and form their spores whilst still enclosed in a cell of their host's tissues, the larger forms very generally are *attached* or implanted, for a certain time, by one end of their bodies in the cell out of which they have emerged, as necessitated by their vast increase of bulk. In other cases (Gregarines velues and egg-parasites) even the larger forms of Sporozoa may remain for a great part of their lives—even up to the formation of spores—as *intra-cellular parasites*.

Two prominent features in the history of the Sporozoa thus sketched are exhibited by *Drepanidium ranarum*, and appear to me to justify us in associating this form with the Sporozoa, whilst at the same time the “wonder” and “astonishment” which the parasite of the Frog's blood has excited, when looked at from the point of view of experimental physiology alone, are definitely put to rest by the association.

The two features in question are the sickle-shape of the *Drepanidium* corresponding with that of the falciform corpuscles of Coccidium and Monocystis, and the cell-parasitism common to *Drepanidium* and the young of Sporozoa.

The agreement of the form of *Drepanidium* with that of the falciform corpuscles of Sporozoa is sufficiently obvious upon comparison of figs. 1 and 4 with fig. 2 c, d. The agreement, however, extends beyond mere superficial form. The size of *Drepanidium* and of the falciform corpuscles of Sporozoa is about the same; the movements of those forms among the latter which have been observed to move, agree with those of the former (see especially Eimer as to the active movements of the falciform young of the Coccidium of the Mouse). Further, although a nucleus has been observed in the falciform corpuscles of some Sporozoa, *e.g.* of *Monocystis lumbrici*; yet according to the accounts of careful observers, no nucleus is to be detected in some of these falciform corpuscles connected with the life-history of other species of Sporozoa. Thus, in the falciform corpuscles of the Coccidium of the House-mouse studied by Eimer, no nucleus has been detected, and I failed to observe one in the falciform corpuscles of *Monocystis thalassemæ*.

Undoubtedly, in assimilating *Drepanidium ranarum* to known forms of Sporozoa, it is to Eimer's parasite of the House-mouse that we should be inclined to point as presenting probably the nearest relationship.

I think it, however, important to draw attention to the close resemblance between the *Drepanidium* of Frog's blood and the

falciform corpuscles which are described as occurring within the spores of a very remarkable Sporozoon—the *Sarcocystis Miescheri*. This organism occurs as a cell-parasite within the striated muscular fibres of such animals as the Pig, Sheep, and Man, and was first described by Miescher, and afterwards (in 1857) by the English anatomist, Rainey. Rainey supposed that it was connected with the life-cycle of *Tænia* and *Cysticercus*. His views as to its significance have been shown to be erroneous, but the parasite has been studied in detail since by other observers, and the correctness of his fundamental observations established. Manz ('Archiv für mikr. Anat.,' 1867, Bd. iii) has given figures of the structure of Rainey's parasite, which attracted some attention in this country at the time of the severe outbreak of cattle plague in 1865, when it was, so to speak, rediscovered by the pathologists who applied themselves to the examination of the meat furnished by diseased cattle. By some writers at that date it was supposed that this organism had a causal relation to the cattle-plague! (See this Journal, vol. vi, New Series, 1866, p. 96.) I have here reproduced a woodcut (fig. 2 A, B) from Leuckart's work, 'Die Parasiten des Menschen,' in order to show the close resemblance of the falciform corpuscles of what I propose to call "*Sarcocystis Miescheri*" with the *Drepanidium ranarum*. The corpuscles are produced in the interior of naked irregular masses (spores), which constitute the contents of the oblong sacs with striated cuticle described by Miescher and Rainey.

*Drepanidium* is not homogeneous in substance. It presents in the living state three bands of differing refractive power, two of higher refractive power placed at the poles of the elongated body and separated by one of less refractive power. When treated with solution of iodine in potassium iodide, the cause of this appearance is seen to be the presence of a strongly refractive oval or rounded particle near each pole of the minute organism (see woodcut, fig. 1). A similar pair of refractive granules are seen in the falciform corpuscles produced in the spores of *Sarcocystis* (see fig. 2 B).

I conclude accordingly—

- 1st. That *Drepanidium ranarum* is a parasitic organism.
- 2nd. That it is probably the young stage of a Sporozoon allied to *Sarcocystis* or to *Coccidium*.
- 3rd. That researches should be directed to the discovery of a Gregariniiform stage, and of cysts containing spores, or of isolated spores in which several *Drepanidia* may be enclosed.

These phases in the life-history of *Drepanidium* are very possibly to be met with in other regions of the Frog's body than the blood-vessels or the spleen.

*Probable Coccidium-phase and Naviculoid Spores of Drepani-*



*dium ranarum*.—It is not at all improbable, though it remains to be definitely demonstrated, that the Coccidium, of which *Drepanidium ranarum* is “the falciform-phase” or “Drepanidium phase” (these terms being applicable to the corresponding phase in all Sporozoa), is already known.

Eimer (‘Psorospermien,’ Würzburg, 1870) has figured (his figs. 48 to 58) a Coccidium from the *alimentary canal* of the Frog, and has traced its division into a number of *spherical* spores.

On the other hand, Lieberkühn, in the ‘Archiv für Anat. und Phys.’ 1854, describes and figures from the *kidney* of the Frog oval spores, which closely resemble in form and size the pseudonaviculæ of a *Monocystis* (Gregarina), such as *M. lumbrici* or *M. terebellæ* (see fig. 5 C, D). The resemblance, indeed, is so close, in the light of Aimée Schneider’s discoveries that we are justified in concluding that the spores observed by Lieberkühn were really spores of Sporozoa. Within the sharply-pointed oval spore-case we observe in Lieberkühn’s figures (taf. i, figs. 4 and 6 of his memoir) delicate, elongate, falciform bodies, two to five in number, disposed just as in the naviculoid spores of *Monocystis lumbrici*, &c. (see fig. 5 A, B and C).

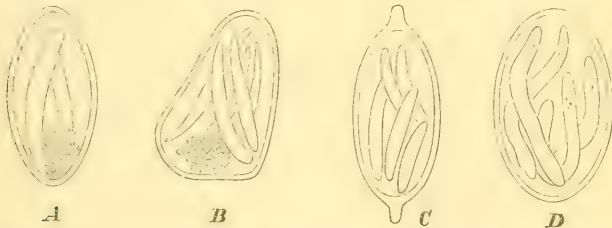


FIG. 5.—Spores (Pseudonaviculæ) of Sporozoa, showing falciform young or Drepanidium-phase within. *A B*. From the kidney of the Frog (after Lieberkühn). *C*. From the Earth-worm (Pseudonavicula of *Monocystis lumbrici*). *D*. From cysts of *Monocystis thalassemæ*.

Lieberkühn did not observe these falciform bodies apart from the naviculoid spore-case, but he describes the active movements of the falciform bodies within the spore-case as “eine langsame Bewegung herauf und herab: sie beugten sich in der Mitte ihres Körpers knieförmig wenn sie an der Spitze des Behälters angekommen waren und kehrten wieder um, gelangten bis an die entgegengesetzte Spitze, krümmten sich wieder und kehrten zur andern Seite zurück.” The spore-case finally burst, and the falciform bodies escaped as spherical corpuscles which elongated themselves again, so as to return to the falciform or rod-like form, and then remained motionless. It appears to be in the highest degree probable that the falciform bodies in Lieberkühn’s naviculoid spores from

*the Frog's kidney were nothing more nor less than our Drepanidium ranarum.* It can be no difficult matter to decide this point by an investigation of the Frog's kidney. Further, it is not improbable that Eimer's Coccidium from the intestine of the Frog is related to the same life-cycle, and is, in fact, the "Gregarina-phase" or "Coccidium-phase" of the same organism which divides first of all so as to form Lieberkühn's psorosperms of the Frog's kidney, whilst these in their turn, give exit to the Drepanidium-phase observed in the Frog's blood and spleen.

At the same time it is possible, from what Lieberkühn observed, that the Gregarina which produces his naviculoid spores is to be found in the kidney also.

*Dr. Gaule's Views as to the nature of Drepanidium.*—Dr. Gaule does not consider *Drepanidium* to be a parasitic organism, but the product of a mysterious metamorphosis of the cells of the Frog's blood and other tissues.

He has published a number of observations as to the abundance of these parasites at various seasons of the year and in different individuals, and as to their occurrence in the spleen, liver, and pancreas. None of these observations are opposed to the view that *Drepanidium* is a parasite, but, on the contrary, are favourable to it. Nevertheless, the notion that *Drepanidium* is a parasite is not seriously entertained by Dr. Gaule at any point in his relation of his observations and experiments; on the contrary, he takes from the first the attitude of one astonished by the phenomena he is describing, and endeavours to account for them quite irrelevantly by suggestions of new and unsuspected physiological processes, by which the protoplasmic units of a complex organism such as the Frog may be converted into forms such as these "Würmschen," endowed with a quasi-vitality. Dr. Gaule's speculations appear to me to be unlikely to commend themselves in any way to those who are instructed not only in the methods of the physiological laboratory, but also in the wider range of phenomena investigated by students of animal and vegetable morphology. Nevertheless they have, on their first appearance, excited considerable interest, which has been largely due to misapprehension, caused by the absence of illustrative drawings from Dr. Gaule's first memoir. When the facts are fully placed before physiologists, the interest excited by Dr. Gaule will give place to regret that speculations so obviously the result of inadequate information should emanate from the celebrated laboratory of Leipzig.

Some of the chief observations due to Dr. Gaule as regards *Drepanidium* are most directly favorable to the view that these minute sausage-like bodies are independent parasitic organisms. I will cite only the following :

1. That they exhibit active movements under circumstances usually favorable to the movements of the Protozoa and Protophyta.

2. That they occur within the cells of the organism in which they are found as well as in its fluids.

3. That they are present in some Frogs and not in others, living under approximately the same conditions.

4. That they vary in abundance in the same Frog, examined at different times.

5. That they are abundant at one time of the year, and not at another.

6. That they are seen on the stage of the microscope to penetrate and enter cells by means of their active movement.

7. That they are also seen to escape from cells by the same activity.

8. That they are localised chiefly in the spleen, though not confined to that organ.

9. That though most abundantly observed in certain specimens of *Rana esculenta* at Leipzig, yet they have also been observed in *Rana temporaria* and in *Triton* sp.

These observations are not merely *consistent* with the view that *Drepanidium* is an independent parasitic organism, but are directly in favour of that view; since they are readily explained if that view be admitted, whilst they remain as isolated and unconnected facts, each requiring a special assumption for its connection with any other theory which may be advanced as to their nature, when the obvious one that they are parasitic organisms is rejected.

The only fact which Dr. Gaule adduces which is inconsistent with the parasitic nature of *Drepanidium* is that in some cells—especially blood-corpuscles—these bodies are *not present when an examination of them is first made on the field of the microscope, and that on the addition to the preparation of .3 per cent. solution of sodium chloride the "Würmschen" are formed there and then in the cells.*

I take the liberty of doubting altogether the accuracy of Dr. Gaule's statements on this point. The supposed fact is not a fact, but an erroneous interpretation of an observation.

It is not so long ago since the view was maintained by accredited physiologists that the *nucleus* of the Frog's red blood-corpuscle did not exist during life, and only came into existence as a *post-mortem* product of commencing disintegration.

We now know very well that there are appearances which favour such a notion with regard to the nucleus. The nucleus is very frequently not visible in the Frog's red-corpuscle during life, and becomes visible as the result of the first changes in

blood removed from the blood-vessels. But the inference to be drawn from this observation is *not* that the nucleus is not present in the living corpuscle, but that the refractive properties of the albuminous matters comprising the nucleus and the body of the corpuscle are such as to make it difficult to define with the microscope the albuminous nucleus embedded in the albuminous body. A change in refractive properties of the substance of either or of both parts of the corpuscle allows us to define the limits of the nucleus.

The same is true with regard to the cell-parasite *Drepanidium*, which (as I have myself observed and as Dr. Gaule admits) becomes visible within a red blood-corpuscle treated with .3 per cent. salt-solution, *just at the same moment and in the same degree* as does the nucleus. In the normal condition of the red blood-corpuscle a minute *Drepanidium*, embracing in crescent form one end of the nucleus, escapes observation—is in fact invisible, just as is the nucleus itself—owing to the refractive index of its delicate substance and that of the body of the corpuscle being identical.<sup>1</sup>

Dr. Gaule's advocate would, however, reply that he has carried his case further than this. He states that taking a drop of blood from a Frog known to yield *Drepanidium* he added at once to it osmic acid or other fixing reagent and that on subsequently staining this blood-preparation though he obtained the nuclei well coloured and well defined—he could detect no *Drepanidia*. On the other hand, a drop of blood from the same Frog—which was *first* treated with a .3 per cent. solution of sodium chloride—yielded plenty of *Drepanidia*, which could be fixed with dilute acid and stained.

In reply to this, I can merely say that I have obtained the *Drepanidia* in Frog's blood and spleen-pulp, which was not exposed to the action of sodium chloride, but was spread when fresh in dilute osmic acid. Further, it is admitted by Dr. Gaule that the *Drepanidia* do exist in the living Frog independently of any experimental treatment of the blood. His differential experiment is inconclusive, because it is possible that one drop of the Frog's blood might contain few or none of the parasites, whilst a second drop (that treated with the salt-solution) might contain many. It is also by no means improbable that a previous treatment with salt-solution, as compared with the immediate treatment of a drop of blood or a piece of

<sup>1</sup> It is a fact that parasitic Micrococci and Bacteria escape observation in the cells of living or fresh tissues in this way, and it is worthy of note that Lieberkühn calls the falciform corpuscles of the naviculoid spores found by him in the Frog's kidney "*diaphanous rods*." They are, in fact, very delicate and transparent objects when alive.



spleen-pulp with a fixing acid (such as osmic or nitric), may so affect the blood-corpuscles and spleen-cells and the *Drepanidia* themselves as to cause the latter to stain more readily in the former than in the latter case.

The mere fact (supposing it to be a fact) that the *Drepanidia* are more readily demonstrated in a preparation which has been treated when fresh with salt-solution than in one which has been treated when fresh with osmic or nitric acid, cannot be admitted as affording any sufficient basis for assuming the existence in nature of a transmutation of organic forms such as that advocated under the name "Heterogeny" by Pouchet and Bastian, and for rejecting the obvious agreements both in form and habits of our *Drepanidium* with the falciform young stages of the Sporozoa or Gregarinida.

*Post-scriptum.*—I think it useful to point out on the present occasion that the views put forward with reference to another parasite of the Frog's blood, viz. the *Trypanosoma sanguinis*, by the same author, Dr. Gaule, whose speculations as to *Drepanidium* have just been discussed, are as novel as the latter. Having given some care to the study of *Trypanosoma* (which I described under the name *Undulina*, believing it to be an unrecorded organism), I am of opinion that Dr. Gaule's views as to that parasite are devoid of justification.

In the 'Archiv der Physiologie,' 1880, p. 375, Dr. Gaule propounds the view, which he illustrates by a plate, that this curious little parasite is a modified colourless blood-corpuscle. Dr. Gaule appears to derive some special satisfaction from launching startling suggestions of this kind based upon the smallest amount of evidence. His ground for concluding that *Trypanosoma* is a modified blood-corpuscle is simply that he observed in Frog's blood abnormal colourless blood-corpuscles vaguely simulating the form sometimes presented by *Trypanosoma*. At every step of his statement of inferences and arguments on this subject Dr. Gaule appears to me to be inconsequent.

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*The MICRO-ORGANISMS which occur in SEPTICÆMIA.* By G. F. DOWDESWELL, M.A., F.L.S., F.C.S., &c. (With Plate VII.)

OUR knowledge of the minute organisms which occur in various infective diseases has been greatly extended during the last few years, since Dr. Robert Koch, now of Berlin, published his investigations on the 'Ætiology of Anthrax or Splenic Fever,' which gave a fresh impetus to this study, and formed a model for subsequent observations, though the actual relations of these organisms to disease are, as yet, far from being definitively settled.

Septic infection, putrid intoxication, or septicæmia, as it has been termed by former writers, is an affection the parasitical origin of which has been warmly contested from the time of the first recorded observations on the subject by Gaspard and Majendie in 1822 and 1823, succeeded by the more systematic investigations of Panum, and subsequently, a continual flow of other writers both in France and Germany, without, however, adding much to the results attained by the latter observer until the last few years, during which the subject has been greatly elucidated. As to the distinction between the different affections formerly included under the terms above mentioned, it may suffice here to say that the disease occasioned by the absorption by the living animal of large quantities of putrid or septic<sup>1</sup> matter, is not a specific parasitical affection, inasmuch as Bacteria, though constantly present in the tissues or organs of the affected animal, are merely so incidentally, and are not the true contagium, the efficient cause of the symptoms; this has been designated by Dr. Burdon Sanderson as septic infection, in distinction to specific septicæmia, a disease which may be occasioned by inoculation with the most minute quantity of septic matter, and which is due to the multiplication and development in the living animal of a minute form of protophyte, and hence is properly a specific parasitical affection. This disease, which I here term simply septicæmia, so nearly resembles anthrax in many respects that to distinguish between them is often difficult, and there is no doubt that the two have been frequently confounded by some of the earlier observers: their statements, too, of the organisms which occur in it are very vague, so that no conclusions can be formed on the subject. It is probable that in many of these

<sup>1</sup> *i.e.* toxical or infective, in which sense the term is now used by pathologists, though originally it merely signified putrescent, in accordance with its etymology.

cases microscopical examination was deferred till some time after death, when various forms of septic Bacteria had developed, which they do in these cases with incredible rapidity.<sup>1</sup> Those organisms which appear to be really characteristic of the disease are so exceedingly minute that they eluded observation till lately demonstrated by the methods adopted and published by Dr. Koch,<sup>2</sup> and by the aid of recent improvements in object glasses. I propose here shortly to describe the microscopical features and the organisms which I have myself observed, following in the main these methods in an examination of the subject.

Septicæmia may be originated artificially in animals, when required for purposes of investigation, by inoculation with a small quantity of decomposing blood; there is, however, as I have found in the investigations of several years, considerable uncertainty in the infectivity of such blood. The recorded experience of many different observers is very discrepant in this respect. I have myself found that it does not generally become infective, *i.e.* capable of originating specific septicæmia, till towards the tenth day, independently of the temperature at which it is kept. The causes which determine this are obscure; though admitting the parasitical origin of the affection, we may suppose that it depends upon the presence and development of the specific organism derived from the atmosphere. The circumstance that such infectivity occurs more frequently in summer than in winter, and that, too, whether the blood is kept in an incubator or at the temperature of the external air, favours this view.

It is requisite that small quantities only of such blood should be used to create infection; one or two tenths of a cubic centimetre (one to three drops) may be used; with larger quantities complications arise and specific infection fails.<sup>3</sup> In the experiments to which these observations refer I have used white mice, which are very susceptible to infection with this disease, as they

<sup>1</sup> Even the latest writer on the subject, A. Krajewski ('Inaug. Diss.,' Dorpat, 1880, reprinted by Professor Semner in the 'Archiv f. Exper. Path.,' &c., for May, 1881) describes and figures various forms of Bacilli and Micrococci in what is regarded as specific septicæmia, but here infection was originated by the injection of considerable quantities of septic matter, 1 to 2 c.c. The same circumstance of delay, renders it very difficult to draw any certain conclusion from the occurrence of Bacteria in the organs of man, where, in ordinary cases, the examination cannot be made for a very considerable time after death.

<sup>2</sup> 'Beit. z. Biol. d. Pflanzen,' v. Dr. F. Cohn, Bd. ii, H. 1; also, 'Untersuch. über d. Ätiol. d. Wundinfections Krankheiten,' Leipzig, 1878, recently translated for the New Syd. Soc. by W. W. Cheyne, M.B.

<sup>3</sup> *i.e.* septic infection, as above defined, occurs, and the subject is killed by the toxical action of the products of decomposition in, generally, a very much shorter time than the incubation period of the parasitical affection.

are to that of anthrax, and the statements here made refer only to these animals.

After infection with putrescent blood, the animal remains without showing any symptoms of disturbance till within a few hours of death; it then becomes dull, remains motionless, closes its eyes, and shortly afterwards dies without further change in about seventy hours, or somewhat more, after infection. From such an animal, if the smallest possible quantity of blood be taken on the point of a lancet or scalpel, and another mouse inoculated therewith in any part of the body, infection occurs with absolute certainty. I have not known one failure in the experiments of several years. Infection established, the disease runs its course in exactly the same way as in the former case, but the period of incubation is materially shorter, only averaging about sixty hours; after, however, the first case of infection by putrid, as distinguished from septicæmic blood, I have not found that there is any increase of infective virulence, as has been previously asserted. I judge of this by the period of incubation<sup>1</sup> which, did any increase of virulence occur, would be shortened, but so far is this from being the case that, in a long series of inoculations of one year's experiments, this period, in the last few of the series, was actually longer than in the first of the same.

Although no symptoms of disturbance are observable in an infected animal till near its end, that is, till upwards of fifty hours after infection, yet I have found that the blood from the living animal becomes infective within eighteen hours after inoculation, and is then as infallibly and actively virulent as that of an animal in which the disease had run its course to its fatal termination; nor does any difference in the incubation period of the infection so established occur.

On the death of an animal from septicæmia, decomposition proceeds with such excessive rapidity, and septic Bacteria appear and multiply so greatly, that no conclusions can safely be drawn from such cases; even in the course of two or three hours very marked changes occur. I have therefore rejected all such, and for this examination have always killed the animal shortly before death would have occurred, as soon as any affection was evident, and another animal has been at once inoculated with its blood to test the occurrence of infection.

In old mice, as probably in all other domestic animals, various pathological changes of the different organs very frequently occur; in these experiments, I therefore generally used young mice. If the organs of one of these are examined after death, the only

<sup>1</sup> Dr. Koch has observed the same thing, and discussed the question, *op. cit.*



changes apparent are a material enlargement of the spleen, which is invariable, and amounts to one third or more of its normal weight; the liver too, in some cases at least, shows incipient degeneration, an atrophy of the hepatic cells, though this change is not very pronounced in the subjects I have examined. The other organs are to all appearance healthy; blood too from the heart, taken and examined under the microscope, appears at first sight normal, the red corpuscles behave as usual, and there is no marked difference in the proportion of leucocytes.<sup>1</sup> On one occasion, however, I observed in the fresh blood of an infected mouse, examined at the temperature of the air, which at that time was between 60° and 70° F., an exceedingly fine filamentous process, in constant undulating movement, evolved from one of the red corpuscles, the diameter of which it exceeded in length; its tenuity was such that under a power of 700 diameters I could not distinguish whether it was truly filamentous or beaded; in appearance it was exactly like a spirillum or vibrio, and I should have taken it for an independent living organism had I not been familiar with the appearance of protoplasmic filaments which under certain conditions are produced from the red blood-corpuscles; these, which I would designate as "Addison's processes," from their first observer, have been fully described elsewhere;<sup>2</sup> their appearance is an indication of a pathological condition of the blood; in normal blood they only occur on the addition of certain reagents or a greatly increased temperature.<sup>3</sup>

Although in the blood and organs examined in the ordinary manner nothing more than above mentioned is observable, when these are prepared by special methods and examined microscopically under adequate powers, it is found that minute

<sup>1</sup> It is, however, deficient in oxygen, as shown by the colour; the true cause of this, with some other points, requires careful investigation. Though the Bacilli obviously take the nutriment which they assimilate from the tissues, the bulk of each one is so excessively small in proportion to that of a red corpuscle, being only at most in the proportion of 1 : 2000, that their direct agency, having regard to their apparent numbers, does not seem adequate to produce this effect. The relative size of the Bacillus in anthrax is widely different. I cannot but think that the appearances in the red blood-corpuscles, described and figured by some writers on this subject, and regarded as pathological, are merely accidental, such as may occur in the preparation of normal blood from, *e.g.* a longer or shorter exposure to the air, or other circumstances.

<sup>2</sup> This Journal, January, 1881.

<sup>3</sup> They resemble very closely in appearance the Spirillum which occurs in relapsing fever, recently shown here by Dr. Vandyke Carter; they are, however, not permanent nor independent organisms, as the latter appear to be.

forms of Schizophytes<sup>1</sup> are present, in some cases in great numbers.

For this examination I have mainly followed the methods introduced as already mentioned by Drs. Weigert<sup>2</sup> and Koch as follows:—A drop of blood is spread in as thin a stratum as possible on a cover-glass, rapidly dried, and strongly heated over the flame, in order to coagulate and fix the albumen; it is then treated for some time with a strong aqueous solution of one of the aniline dyes, which is drained off, the preparation rinsed with alcohol, either dried or treated with oil of cloves, and mounted in Canada balsam. I have not found the use of the oil of cloves necessary; it decolorises the Bacilli, which are never too deeply stained. If the blood is in a sufficiently thin stratum this is all that is requisite. The Bacilli which occur in these cases are so characteristic that when sufficiently stained it is impossible to mistake them, or to confound portions of the tissues with them. The preparations may, however, be treated for a very short time with a weak solution of acetic acid (one per cent.) or of potassic carbonate (five per cent), preferably the latter, for I have frequently found the former decolorise the Bacilli almost instantaneously; in either case the preparation is then washed first with water then in alcohol and mounted in Canada balsam.

To prepare the different organs for microscopical examination on the animal being killed, they are at once excised and placed in strong alcohol.<sup>3</sup> When sufficiently hardened sections are cut; they are then stained by immersion for some time in a strong aqueous solution of one of the aniline dyes. It is then found that any Bacteria in the preparation and the nuclei of the tissues are alike stained with little distinction, but by washing in water and treating with a solution of sodic or potassic carbonate the tissues become pale, and the staining of the nuclei fades, while the Bacteria, having a stronger affinity for the aniline colours, are less affected, and thus strongly differentiated. The strength of the alkaline solution and the length of time the

<sup>1</sup> Bacteria is the general, though inconvenient, term here for the whole group of these organisms, otherwise named Microzymes, Microphytes, or Protozymes; in Germany, Schizophytes or Schizomycetes; in France, Bacteridie, and various other names; following F. Cohn, they are now generally divided into three principal genera: Bacteria, Bacilli, and Micrococci.

<sup>2</sup> Koch, loc. cit.; Weigert, 'Virchow's Archiv f. Path. Anat., &c.,' for May, 1881.

<sup>3</sup> If absolute alcohol is used, the red blood-corpuscles are preserved in the vessels, by which a natural injection, as it were, is made; and in many cases, especially in exceedingly thin sections, the preparation is much improved; of less strength than about 90 per cent., alcohol dissolves the colouring matter of the corpuscles.

sections are left in it must depend upon their thickness, the depth of staining, &c. I have used solutions of from five to ten per cent. of either salt, for from fifteen minutes up to two hours. I have not found treatment with acetic acid successful with sections of the organs any more than with the preparations of blood. After this the section is washed in water, treated with alcohol and oil of cloves for a few seconds, and mounted in balsam.

In preparations of the blood of a septicæmic mouse, made as above described and successfully stained; there is seen in some cases a minute form of Schizophyte, a *Bacillus*, the greatest length of the individual rods of which is about  $1.6 \mu$  ( $\frac{1}{15000}$  in.); the breadth is somewhat too small to measure directly, but does not exceed a fifth of the length, *i. e.* about  $0.3 \mu$  ( $\frac{1}{30000}$  in.). They are mostly single; sometimes in pairs united endwise, lying amongst the red corpuscles; they are not found within the white corpuscles, nor are they, to my observation, very numerous in blood from the heart. In preparations of the lungs of the same subjects, these *Bacilli* occur sometimes in great numbers, both within the capillaries and in the tissues on the alveolar septa. Here convoluted filaments or chains composed of several individual rods or articulations are found. It is therefore a true *Bacillus*, of a form not, I believe, hitherto described. Though minute, it may be readily resolved by a good  $\frac{1}{8}$ th objective. Fig. 2 in the accompanying plate represents its size and form. In some of the same preparations of lung there also occurs another organism, of comparatively gigantic proportions, morphologically identical to all appearance with the now well-known *Bacillus anthracis*,<sup>1</sup> or the hay *Bacillus*. It is shown in the lower part of the same, Fig. 2. It here forms long convoluted filaments, the articulations of which are of considerable length and granular, showing an early stage of development. Its breadth is about  $1.0 \mu$  ( $\frac{1}{25000}$  in.). They are not numerous; I have not found more than two distinct filaments in the same section, and those in which they occur are all from the same subject—a young mouse, previous to inoculation apparently healthy.

There is also found in the blood in other cases, another ultra-minute form of Schizophyte. In length it does not exceed  $1.0 \mu$  ( $\frac{1}{25000}$  in.), in breadth  $\frac{1}{7}$ th, or at most  $\frac{1}{6}$ th, of the length, *i. e.* less than the  $\frac{1}{15000}$ th in.<sup>2</sup> It occurs singly or in pairs amongst the red corpuscles, and also, sometimes thickly crowded,

<sup>1</sup> Sometimes pronounced anthracis, but wrongly, and which wounds the ear of any one brought up in the fear of the “*Gradus ad Parnassum*,” the penultimate is distinctly short both in Latin and the original Greek.

<sup>2</sup> In comparing micrometric measurements by different observers, varia-

within the white corpuscles, which are considerably swollen and altered. It also is a true Bacillus, sometimes, though not very frequently, forming moderately long filaments of several articulations, which, from their mode of union, must possess flagella; though these would be of inestimable tenuity, the Bacilli themselves verging on the confines of the invisible with the very best optical appliances and arrangements, and being, I believe, the most minute independent organisms yet described. In the lungs this Bacillus is also found, sometimes in vast numbers, but confined to the blood-vessels, both the larger vessels and the capillaries. Here in a successful preparation the deeply-stained white corpuscles filled with these organisms are readily seen. The Bacilli are also found lying thickly on the inner walls of the vessels; but to resolve the individual forms distinctly requires a magnifying power of 12,000 or 14,000 diameters at least, and good illumination. The general appearance which they present is shown in the accompanying plate (fig. 1), which is an accurate representation of a section of lung which has been treated with a strong solution of sodic carbonate after the steps above described. This has rendered the tissues pale and somewhat indistinct, while the deeply-stained masses of Bacilli within the white corpuscles are conspicuous in the different blood-vessels; but the amplification in the drawing is not nearly sufficient to show the individual forms on the walls of the vessels.

The relative size and form of these Bacilli is given in fig. 3, as shown under a magnifying power of nearly 3000 diams. This is the organism described and figured by Dr. Koch as *B. septicaemiae*, and regarded by him as the specific infection, the true contagium of the disease; and as he very kindly gave me some of his own preparations during his recent visit here, I have been enabled directly to compare the organisms which occur in both his and my own, and find them identical.<sup>1</sup>

For staining these preparations a solution of methyl aniline tions in the standard employed must be borne in mind. I have found considerable difference between the scales of different makers which I have. Hitherto there has been no standard micrometer scale for reference; one, however, has now been completed by Professor Rogers of Cambridge, U.S.A., of which microscopists will no doubt largely avail themselves.

<sup>1</sup> These minute organisms form an excellent test for any high power objective, but I may here mention that the best possible independent object, as distinguished from a mere line or marking, is a minute barb on the point of the head of the spermatozoon of the newt (*Triton cristatus*), well known as possessing a large and conspicuous "filament." This barb was first observed and shown to me by Mr. Nelson, the accomplished diatomist of Marlborough Hill, St. John's Wood, to whom I am also indebted for assistance in the measurement and examination of these most minute microscopical objects.



violet has generally been recommended ; that hitherto procurable in this country I have found very fugitive and uncertain in use, the staining of the section, even after prolonged immersion, frequently fading entirely upon a short treatment with alcohol, and the "gentian violet," lately recommended by Weigert (*loc. cit.*), I have found in comparative trials even more fugitive, its colour being altogether lost by merely washing with water. Mr. Martindale, however, of 10, New Cavendish Street, W., has lately supplied me with some of the former salt procured from Germany, which I have found much superior and less fugitive. The preparations in which the organisms here described are found are all from young mice, previous to artificial infection healthy, killed and treated by the methods already described. I have not found the occurrence of these Bacilli constant in all such-subjects,<sup>1</sup> and in other organs than the lungs and in the blood, I have not observed them. The organism in question here, however, is so excessively minute that the possibility of demonstrating it depends entirely upon the methods employed in preparing the specimens, and their differentiation by successful staining from the surrounding tissues. The action of the aniline dyes, too, is always somewhat uncertain and capricious ; it has been before remarked that the chemical reactions of Bacteria vary in different organs of the same animal, and I have observed that in the case at least of the liver the bile salts interfere with their staining ; negative observations, therefore, especially in respect to this organism, are worth very little. From my own observations in other cases, however, it seems to me probable that if the lungs are, as they seem to be, the principal nidus of the parasite, it would not during the life of the animal infest its other organs, but would invade them very rapidly upon its death.

I have not succeeded satisfactorily in cultivating this *Bacillus* artificially ; its ultra-minute size renders this a matter of especial difficulty. By microscopical examinations of the fluids or media employed, it is impossible to ascertain whether the organisms, if unstained, are present or not.<sup>2</sup> The cultivation is said to have been accomplished by others, though the details are not published, and until this is done strictly, as in the case of anthrax, by Dr. Koch, of pneumo-enteritis in swine, by Dr. Klein, and some few other cases, its relation to the affection in which it

<sup>1</sup> Dr. Koch, on the other hand, finds them invariably present. On this point principally turns the whole question of the ætiology of this affection.

<sup>2</sup> In examining or searching for Bacteria, I have usually found that if they are *unstained* the use of a sub-stage condenser, however the diaphragm may be manipulated, tends considerably to obscure them, more especially with high powers, and in the case of even such a comparatively conspicuous and large organism as *B. anthracis*.

occurs cannot be regarded as conclusively shown. I have found quite recently that living Bacteria may be stained by a solution of Bismark brown, which does not appear to have a toxical effect upon these organisms as other aniline dyes have; by its action spores developing within the Bacilli are conspicuously differentiated. By this means, in conjunction with the method recently described by Dr. Koch of adding gelatine to the nutrient fluid, their cultivation and further study will no doubt be facilitated.

I have not succeeded in transmitting specific septicæmia by inoculation from one animal to another of a different species, notwithstanding numerous experiments. Dr. Koch, I understand, has done so, and finds in the blood of pigeons infected from another animal a totally distinct form of Schizophyte, a small micrococcus of characteristic appearance, morphologically identical, I believe, with the microbe of Pasteur's cholera des poules, and which Dr. Koch regards as the specific contagium, the true cause of the disease. This, however, appears to me to involve a direct contradiction, or the occurrence of distinct heterogenesis in the Schizophyte, for the doctrine that the various forms of these organisms are but different phases of one and the same species, modified by external conditions, though a view which is supported by the high authority of von Nägeli, yet lacks experimental evidence. Others, again,<sup>1</sup> have recently asserted that by cultivation in different media, through several generations, a transformation of physiological species has been effected, so that what is at one time a perfectly harmless form, and universally diffused, becomes the virulent contagium of infective disease. These results, though accepted by some, as by Prof. Virchow, are regarded as erroneous by a very high authority on this subject, M. Pasteur, whose brilliant results, together with those of M. Toussaint, in France, and others,<sup>2</sup> on the methods of preventive inoculation, now well known, have lent a new and practical importance to the subject, and demonstrated a possible beneficent function of these organisms, more

<sup>1</sup> Dr. Hans Büchner, in the 'Sitz. Bericht. d. math. phys., Classe d. K. B. Akad. d. Wissens. zu München,' H. iii, 1880, asserts the transformation in artificial cultivation of the *B. anthracis* into the hay Bacillus, and also the converse change. Ingenious and laborious as his methods are, they are defective, and the admitted occurrence of contaminations throughout, in the cultivations, on the purity of which the whole question at issue depends, entirely invalidates his conclusions. Dr. Grawitz, in 'Virchow's Archiv,' Bd. 81, and in some previous journals, records experiments with the Hypomycetes to a similar effect.

<sup>2</sup> In this country, Dr. Burdon Sanderson and Mr. Duguid, 'Journ. R. Agric. Soc.,' 2 ser., V. xvi; Dr. Greenfield, 'Proc. R. Soc.,' V. xxx, p. 450; and 'Journ. R. Agric. Soc.,' 2 ser., V. xviii, pt. 1.

than compensating for the increasing number of affections in which they are shown to be present, and which are attributed by some to their causation.<sup>1</sup> Thus it may well be said that the study of these organisms has acquired a vastly increased and unexpected importance.

The observations here recorded have been made in the course of a more extended investigation on septic and other infective diseases, carried on during some years past at the Brown Institution, Wandsworth Road.

*August, 1881.*

<sup>1</sup> The latest instances are, in malarial fever, Klebs and Tommasi Crudeli, 'Atti d. Accad. d. Linc.,' v, f. 1, December, 1880. J. Braulicht, 'Virchow's Archiv' for April, 1881, found in tap water, during an epidemic of typhoid fever, a minute Bacillus, apparently identical with that previously observed in the blood of typhoid patients by Klebs and Eberth, and which, when cultivated, was found to be infective. More particularly too, in tuberculosis and serofulous affections, as recorded in an important work published at Stuttgart last year by Dr. Max Schüller; to which may be added the Spirillum of relapsing fever, which was demonstrated very completely by Dr. Vandyke Carter at the late International Medical Congress, previous accounts of which had been somewhat imperfect.

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PRINGSHEIM'S RESEARCHES *on* CHLOROPHYLL.<sup>1</sup> Translated and condensed by Professor BAYLEY BALFOUR, of Glasgow University. (With Plates VIII and IX.)

For the past two or three years the author has been engaged with an investigation of the character of chlorophyll-corpuscles, of the action of light upon them, and the function performed by chlorophyll in the plant. From time to time his results have been published, and these are now all brought together in the paper, copiously illustrated, which is here presented. The papers on the subject previously published by the author are,—

1. "Ueber Lichtwirkung und Chlorophyllfunction in der Pflanze." 'Monatsb. d. Berl. Akad. d. Wiss.,' July 1879.
2. "Ueber eine Methode microscopischer Photochemie." 'Verhandl. d. Bot. Ver. d. Prov. Brandenburg,' July 1879.
3. "Ueber das Hypochlorin und die Bedingungen seiner Entstehung in der Pflanze." 'Monatsb. d. Berl. Akad. d. Wiss.,' November 1879.
4. "Remarques sur la Chlorophylle." 'Comptes Rendus,' xc, Jan., 1880.
5. "Zur Kritik der bisherigen Grundlagen der Assimilationstheorie der Pflanzen." 'Monatsb. d. Berl. Akad. d. Wiss.,' Feb. 1881—

and they may be consulted for details regarding the experiments.

In this communication the subject is treated of in the seven following sections.

- I. Structure and Composition of Chlorophyll-Corpuscles.
- II. Method of Microscopical Photochemistry.
- III. Action of Light. Paralysis and Death from Light.
- IV. Respiration of Green Tissues in Light, and the Function of Chlorophyll.
- V. Assimilation and Colour.
- VI. Formation of Hypochlorin in Young Seedling Plants and its Relations to Assimilation.
- VII. Some Details of Experiments.

<sup>1</sup> 'Untersuchungen über Lichtwirkung und Chlorophyllfunction in der Pflanze.' Von N. Pringsheim, S. 152, mit 16 lithographirten Tafeln. Leipzig, 1881.



*I. Structure and Composition of Chlorophyll-corpuscles.*

In micro-chemical investigations of chlorophyll-corpuscles hitherto made a solvent has been generally used for separating the colouring matter from the ground-substance of the corpuscle, a method possessing the disadvantage that other substances besides the colouring matter are extracted in the solution from the corpuscle, and these have not been sufficiently distinguished and separated from the colouring matter. In the investigations recorded in the following pages, a new method, against which such objection cannot be raised, has been employed. It consists in warming green tissues in water or subjecting them to the action of steam or treating them with dilute acids. Different effects are produced according as one or other, or a combination of these agencies, is made use of; but they coincide in this general effect, that they cause the colouring matter, along with certain fluid or semifluid substances accompanying it, to exude from the chlorophyll-corpuscles in the form of larger or smaller drops, while these coat the periphery of the decolorised skeleton or ground-substance which itself retains the original form of the corpuscle.

If a portion of green tissue be warmed for fifteen minutes to an hour in water of a temperature of  $50^{\circ}$  to  $80^{\circ}$  C., or if it be suspended during fifteen minutes to several hours in a flask, so as to be in contact with the steam of boiling water (the degree of temperature and the time required for the action varies with the tissue taken; in most cases boiling the tissue for five minutes is the quickest and most convenient procedure), viscid drops of varying size become visible at the circumference of the chlorophyll corpuscles. They may be watched exuding from the substance of the corpuscle, in number depending upon the duration of the operation. Always coloured, they are usually chlorophyll-green, but this may be brighter or darker, and the tint in some cases is yellow or blue-green, occasionally olive-green, more seldom reddish-brown (figs. 1 and 3). They are completely soluble in alcohol and ether, and consist of the colouring matter with an oily basis, which is the vehicle holding it and the substances accompanying it in solution.

In proportion as the exudation proceeds, the ground-substance is decolorised and the colouring matter may be completely removed from it. The structure of the ground-substance then becomes visible, and it appears under a high magnifying power as a skeleton, having the shape of the original chlorophyll-corpuscles, composed of a soft sub-

stance differentiated to form a hollow body, the circumference of which is abundantly and uniformly pierced in a sieve-like manner, It has, in fact, the character of a hollow sponge. It is difficult to accomplish complete decolorisation by heating in warm water alone, and this is best and most easily brought about by a combination of warmth and dilute acids. The character of the tissue must be taken into account, as, for different tissues one or other method may be more favourable. This character of the ground-substance becomes evident if the corpuscle be decolorised by alcohol or other solvent, and also, as will be presently described, by the action of intense light (fig. 26); and the fact that the operation of these different agencies demonstrates the same structural characters in the decolorised chlorophyll-corpuscles is strong evidence that this is also the structure of the corpuscle under normal conditions. Chlorophyll-bands, plates, and masses show equally well this structure.

These, the normal effects of heating a tissue in water or exposing it to steam, may be complicated by the temperature being so high as to affect the starch contained in the chlorophyll-corpuscles, and by causing the granules to swell and rupture the corpuscle thus destroy its form. All tissues are not equally sensitive in this way. Some may be subjected for hours to the action of water at a high temperature, or to steam, without the chlorophyll-corpuscles losing their structural character, whilst in others a few minutes suffices to cause them to lose their form and to coalesce; or, and perhaps more usually, whilst retaining their individuality, they swell internally and rupture, and then appear as irregularly burst hollow spheres (fig. 3). The amount of starch present in the chlorophyll-corpuscles determines to a certain extent the rapidity of this effect. The more starch present the more certainly and the more easily will it be produced; but there are many tissues in which starch is present and yet there is complete immunity from this action. This rupturing of the chlorophyll-corpuscles, due to the starch, must be distinguished from the exudation of colouring matter and its accompanying substances already mentioned, which is quite independent of the starch content, and is brought about at a much lower temperature.

The exudation of the coloured drops appears to be the mechanical effect of a simple swelling of the ground-substance due to its absorbing water, and it thereby exercises a pressure upon and squeezes it out, the vehicle holding the colouring and other matters in solution which fills its meshes.

The effect of treating green tissues with dilute acids is somewhat different, and has led to the discovery of a universally present constituent of the chlorophyll-corpuscles,—hypochlorin. Hydrochloric acid, in the proportion of 1 vol. to 4 vols. of water, is the most favourable for the detection of this substance. But others may be used, *e.g.* sulphuric acid, 1 vol. to 20—40 vols. of water; glacial acetic acid, 1 vol. to 2—4 vols. of water; picric acid, 1 vol. to 3—6 vols. of water; these, however, require much care in application, and are less certain in their effect than hydrochloric acid, which has been chiefly used in these investigations. Glacial acetic acid, 1 vol. to 2 vols. of water, is specially favourable for bringing out the perforated wall-structure of the chlorophyll-corpuscles.

In specimens preserved for some years in Hantz's fluid, in dilute glycerine, and in chloride of calcium, hypochlorin was observed to have exuded from some chlorophyll-corpuscles.

If a green tissue be treated with dilute hydrochloric acid, a sudden change in colour is the only immediate effect apparent; the whole tissue, like the chlorophyll-corpuscles in the cells, acquires a yellow-green, gold-yellow, or brownish tint. No decomposition of the green colouring matter has taken place, nor is it dissolved by the acid, and the chlorophyll-corpuscles are unchanged in form and structure. But after a few hours there appear in the substance of, but chiefly near the periphery of the chlorophyll-corpuscles, dark reddish-brown or rust-coloured, soft, greasy masses, hardly of the nature of drops, being of irregular form, and sometimes showing a firmer limiting layer (fig. 4) or pellicle. These, which are distinguished by their larger size, irregular form, colour, and denser consistence, from the drops exuded after warming with water, appear invariably in all plants, whatever their position in the vegetable kingdom, and whatever be their habitat, whether land, fresh or sea water, that contain chlorophyll, be it attached to isolated chlorophyll-corpuscles or to variously shaped masses, and they appear equally in those which contain starch or oil in their corpuscles and in those which have no starch. The following are some of the plants in which these have been specially observed:—Amongst *Algæ*, species of *Ulothrix*, *Draparnaldia*, *Chaetophora*, *Aphanochaete*, *Coleochaete*, *Mesocarpus*, *Spirogyra*, *Cladophora*, *Oedogonium*, *Bulbochaete*, *Closterium*, *Micrasterias*, *Pediastrum*, and in *Enteromorpha*, *Cladophoreæ*, from the German Ocean. Amongst mosses, in the protonemata, leaves and stems of *Mnium*, *Hyppnum*, *Fontinalis*, and *Sphagnum*. Amongst vascular cryptogams, in pro-embryos of *Pteris*, *Blechnum*, and

*Gymnogramme*, and in leaves of species of *Selaginellæ*. Lastly, amongst Phænogams, in leaf tissues of *Taxus*, *Zostera*, *Vallisneria*, *Elodea*, *Ceratophyllum*, *Heliconia farinosa*, *Tilia*, and *Ampelopsis*. This effect is not observed in plants that are not chlorophyll-green, e.g. in *Phycochromaceæ*, *Diatomaceæ*, *Phæosporeæ*, *Fucaceæ*, *Florideæ*, and *Fungi*,—traces of it only were observed in some stages of their life-history in diatoms,—although the exudation of colour drops after warming in water may be observed. The *Vaucherieæ* are a curious exception, as in them frequently no trace of the action is visible.

After a short exposure to the acid, in from six to sixty hours, the rust-coloured masses begin to change in appearance. They become angular and spikey, and form on the surface of the corpuscle more or less broad scales or nests, with an indistinct crystalline texture, from which sharp-edged and pointed prolongations develop (figs. 2 *b* and 5), or they form cloud-like precipitates with firmer embedded pieces (fig. 2 *a*). At a later period still further changes take place, and these go on equally well if the specimen be left in the acid or removed to pure water. From the irregular masses are developed short, stiff, straight (often rhombic), or crumpled or wavy rods, like the so-called rods of the waxy coating on *Musaceæ*, having blunt or pointed ends (figs. 15 and 16); or the productions are long, firm, straight, or coiled needles (figs. 6, 7, and 17); or delicate flexible filaments (figs. 8 and 9). No differentiation is observed in these crystalloidal masses, except occasionally in some of the rods, when, owing to a slight condensation of the periphery, a double contour is shown.

As the rust-brown masses first appear within the periphery of the chlorophyll-corpuscle, they have the appearance of confluent oily drops, in consistence, outline, and movement, resembling a volatile rather than a fatty oil. And the subsequent formation from them of the crystalloidal processes probably results from the conversion, at least in part, of the oily substance into resin. The whole of the exuded mass does not assume the crystalloidal form, but there is always a remnant of the original substance left like a mother liquor, out of which the crystalloidal bodies have solidified. There is, then, here a mixture of substances with which the colouring matter of the chlorophyll-corpuscle is in part incorporated, which being insoluble in water, dilute salt solutions, and acids, and completely soluble in alcohol, ether, benzol, bisulphide of carbon, petroleum, volatile oils, &c., must be referred to the group of resins and fats. The colour of the



mixture depends upon the modified chlorophyll, for the more highly-developed crystalloidal processes, if exposed for a short time to light, change colour and get lighter, often bleaching slowly from the point backwards, and in this way may become quite colourless (fig. 7). The crystal-like projections and the short rods are often from the first destitute of colour.

It is that constituent of these extruded masses which forms the crystalloidal bodies, or rather the form in which it pre-exists in the chlorophyll-corpuscle, that is termed hypochlorin. Is it an independent constituent of the chlorophyll-corpuscle, or is it derived from the green colouring matter by the action of the acid?

Were it a product of the action of the acid on the green colouring matter its presence should be indicated in every chlorophyll-corpuscle when treated with the acid, for there is no reason to doubt that the characters of the chlorophyll-corpuscles in a normal tissue, in one and the same cell at least, are essentially alike. But this is by no means the case. Often the crystalloid-bodies develop only upon a few chlorophyll-corpuscles in a cell (fig. 7), and are absent from the others, or they may be entirely absent from one or more cells in a tissue otherwise rich in the substance. Again, in the majority of *Algæ* where the chlorophyll is attached to masses of various forms, whilst hypochlorin is rarely entirely absent from a cell or from a filament, the amount present varies much both with the season and with the stage of development of the cells.

This sporadic distribution of the hypochlorin, while it indicates the existence of this substance as a general constituent of the chlorophyll-corpuscles, independent of the colouring matter, points also to its function as a formative material, like starch and oil, in the chlorophyll-corpuscle, and its detection will depend upon whether it has accumulated or been used up in the chlorophyll-corpuscle; the amount discoverable at any time representing excess of supply over demand at that particular moment.

In plants with chlorophyll-bands or plates, further evidence of the presence of hypochlorin as a distinct and independent body is afforded by definite anatomical relationships of the crystalloid outgrowths to the chlorophyll-masses. In *Spirogyra*, for example, they appear at widely and almost equally distant points on the edge or middle line of the bands (fig. 10). The same is observed in those *Edogoniæ* that possess chlorophyll-bands, and in *Draparnaldia* the crystalloids, always few in number, occur on the crossing

chlorophyll-bands (fig. 9). In all cases, too, they appear preferably at the periphery of the amylum-bodies in the bands, and before starch is visible in these (figs. 15, 16, and 17). So universal is this that any spot where hypochlorin is observed not in relation to an amylum-body may be assumed to be a seat of election for one. The constancy of the appearance of hypochlorin on the periphery of the amylum-bodies, suggests a genetic connection between them, and seems to indicate that hypochlorin plays an important part in the nutritive function of green cells.

An anatomical fact, hitherto unrecognised in the organisation of *Spirogyra*, may here be noticed. The threads of protoplasm extending outwards from the central plasma mass in each cell do not, as was supposed, end in the general protoplasmic lining of the cell wall, but each passes directly or by its branches to the internal surface of a chlorophyll-band, and there dilates in a trumpet-like manner and grasps, as it were, an amylum-body (fig. 14). If, as sometimes occurs, there is no amylum-body visible at the point where the thread is in contact with the chlorophyll-band, the spot may be considered one where such a body will subsequently appear. As the amylum-bodies increase by division, the grasping protoplasmic thread also divides by forking, and thus each daughter amylum-body is grasped by a protoplasmic thread; and, on the other hand, the protoplasmic threads may divide in the first instance, and a new amylum-body is subsequently formed in the chlorophyll-band at the extremity of the new protoplasmic thread. As an outcome of this mode of increase, the adjacent amylum-bodies are often connected bridgeways by threads of protoplasm; and as longitudinal division of the chlorophyll-bands often proceeds synchronously with the multiplication of the amylum-bodies and the forking of the protoplasm threads, the amylum-bodies so connected may be in different spires of the chlorophyll-band. In the angles of the forks of the branching protoplasmic threads there is usually visible in strongly growing *Spirogyra* filaments a thickening of the substance of the thread in which a vesicle, perhaps a kind of amylum-body, lies. There is, then, in *Spirogyra*, a direct connection through the threads between the amylum-bodies themselves, and also between them and the nucleus.

These very definite anatomical relationships render *Spirogyra* a very favourable subject for future investigation of the condition in which hypochlorin exists in the fresh cell when unacted on by acids. It is not, indeed, difficult to observe in its chlorophyll-bands before they are treated with acid,

signs betraying the existence of some peculiar substance. If a cell of *Spirogyra crassa*, or other large species rich in hypochlorin, be slightly injured, either by mechanical pressure or by warming (up to  $30^{\circ}$ — $40^{\circ}$  C. according to the species), so that but little disturbance of cell content is produced, there are seen in the projections at the edge of the chlorophyll-bands and beside the amyllum-bodies, that is, at the exact position where hypochlorin becomes visible when an acid is employed, large clear vacuole-like spaces filled with strongly refractive oil-like matter (fig. 13). These, which have not been hitherto generally noticed, differ from the small fat-particles abundantly distributed through the bands by their larger size, more fluid content, and the possession of a limiting pellicle. A slight contraction of the band in breadth is associated with their appearance, and the projections on the edge of the band disappear. After very slight warming the large vacuoles may be readily observed at the edge of the band to rupture their skin, and their fluid content is disseminated in the surrounding protoplasm lining the cell wall (fig. 12). In large species of *Spirogyra* a spontaneous coalescence of the vacuoles may often be witnessed.

The position of these vacuoles leads to the conjecture that it is their oily content which forms the hypochlorin excrecences, a conjecture quite conformable with the easy destructibility of hypochlorin in green tissues. For this accumulation of oily matter in vacuoles, the ready escape of the same, and its dissemination in the protoplasm under slight mechanical or thermal influences, affords an explanation of those cases where the hypochlorin reaction is absent or suppressed, and further explains the results, now to be described, that follow when green tissues previously warmed in water are treated with dilute acids. After such treatment the hypochlorin reaction is suppressed.

If a tissue which has been warmed in water, and in which the characteristic exudations have been developed, be treated with dilute hypochloric acid, no hypochlorin masses are formed, neither on the coloured drops nor on the ground-substance of the chlorophyll-corpuscle, provided that the duration of warming and the temperature have been sufficient for the tissue and species examined. Usually the only visible effect is a slight change to a browner tint of the coloured drops. The time and temperature limits vary considerably with the species. Thus, for *Mesocarpus scalaris*, which is very rich in hypochlorin, five minutes' exposure to a temperature of  $42^{\circ}$ — $43^{\circ}$  C. suffices; *Cladophora* requires five to

fifteen minutes at 50° C.; in *Spirogyra*, *Ulothricheæ*, *Coleochaetææ*, *Edogoniææ*, and their allies, a temperature of 45°—50° destroys the reaction. For plants with isolated chlorophyll-corpuscles, such as *Chara* and *Nitella*, and soft-leaved plants as *Elodea*, *Callitriche*, &c., a quarter to half-an-hour in water of 50° is enough. *Fontinalis* also takes fifteen minutes at 50° C. In *Mnium*, fern embryos, *Selaginella*, and in *Vallisneria*, a longer time at 50° C. is required, or the temperature must be raised to 60°—80° C. Boiling or steaming brings it about more rapidly. Fifteen minutes steaming is enough, as a rule, though at times half-an-hour or an hour is wanted. It would appear, that in all these cases the hypochlorin is destroyed by heat, and vanishes without damage to the colouring matter or destruction of the chlorophyll-corpuscle or mass itself.

Other hurtful influences, which only to a slight extent change the normal character of the cell-content, destroy the hypochlorin without there being any visible change in the chlorophyll-corpuscle itself. Cells in such an abnormal or unhealthy state occur frequently in cultivated specimens of *Spirogyra* or of *Nitella*. They can easily be produced artificially if the conditions of life are made unfavourable. If a cell of *Spirogyra* be injured mechanically, or if the conditions for its existence are not suitable, the first sign of unhealthiness is seen in the chlorophyll-bands, which lose their outline, contract, and if the conditions be continued disintegrate to formless particles. Such signs are very frequently indication of a faulty nutrition only. The colour of the bands is not in the least affected, and the amyllum-bodies and oil-drops remain undestroyed. Many cells in a filament may be quite healthy, and others may show all stages of commencing sickness. On treating with hydrochloric acid, the healthy cells alone show hypochlorin, none is found in the unhealthy cells, or only a slight trace when the diseased state is not very pronounced. It is possible that some of the cases already referred to where one or more cells in the midst of a tissue rich in hypochlorin show no trace of this substance, and in which the hypochlorin was supposed to have been completely used up in the nutritive process, are instances of disease. The cells, though not visibly so, may be really in an abnormal condition, which has resulted in and is made known by the loss of their hypochlorin.

Associated, then, with the colouring matter, hypochlorin is a substance universally present under normal conditions in chlorophyll-corpuscles, whatever their shape. These having



the structural character of a hollow sponge or network, their meshes are permeated by the hypochlorin and the oily vehicle with the colouring matter, which can, by the methods mentioned, be readily extracted. In the interior, too, of these hollow perforate chlorophyll-corpuscles, the secondary deposit of starch and such like formative material takes place. The whole organisation of the chlorophyll-corpuscles is thus admirably suited for the performance of their function, and they are peculiarly fitted for absorbing and condensing gases.

## II. *On the Method of Microscopical Photochemistry.*

This method has been designed with the view of directly observing under the microscope the action of light on the contents of plant-cells, and of determining the light and heat absorption in the several elements of the cells and the activity stirred up within the tissue by the sun's rays. It is complementary to and an extension of the analytical methods hitherto employed for determining gas interchange in white and in coloured light, and it permits of a more correct and complete explanation of the experimentally-known facts of the respiratory process in plants. For whilst the most accurate quantitative and qualitative estimation of inspired and expired gases gives no clue to the share which the individual cell-elements take in the respiration, the observation under the microscope of the action of light in the cells, in circumstances which promote a rapid effect, enables the changes taking place in the elements, as well as the conditions under which they are, or are not, brought about, to be carefully studied.

A high light intensity is necessary to produce quickly the desired effects. Experiments upon plants with intense light are greatly required, especially with reference to the relative energy of the different rays of the spectrum in gas-interchange. Plants growing behind coloured screens are evidently in relative darkness, varying with the colour, as compared with normal conditions, and as researches into this subject have all been conducted after this method, the results are only valid for low and insufficient intensities. More particularly is this the case with the so-called chemical rays. These blue and violet rays are usually considered as being active only in heliotropic phenomena, and as having little or almost no effect in gas-interchange of plants. But all coloured screens (blue glass and sufficiently concentrated solutions of ammonia copper sulphate) used to produce monochromatic blue or violet light, are very dark. They allow in diffuse

daylight, and in ordinary sunlight, only a feebly intense light to pass, which has much less effect than the same rays have when acting directly in sunlight. By the yellow and green screens employed (green glass or solutions of copper chloride and potassium bichromate) the intensity of the light is diminished to a much less extent. The intensity of light action is, in experiments with coloured screens, undoubtedly dependent upon the illuminating power of the acting rays, and the strikingly small effect given as the result of the operation of blue light is to be explained in great part by the non-transparency of the screen employed. Moreover, as the blue are preferably and more strongly absorbed in the chlorophyll than the green and yellow rays, they will act with less intensity than these upon the cell contents protected by the chlorophyll (that chlorophyll plays this part is one of the results of the researches here described), and it is necessary to bear this fact in mind when considering the effect of, and in estimating the relative energy of rays of different colour. In all experiments with blue rays the specific chlorophyll-absorption must be compensated by using more intense light.

In studying photochemical effects under the microscope, the simplest method is to concentrate sun's rays upon the object by means of a condensing lens under the stage, care being taken that the object is kept accurately in the plane of intense illumination. In the researches recorded here a microscope with a double stage was used, the upper one for the object vertically movable, the lower one fixed, and provided on the under side with a doublet-condenser of plano-convex lenses, which gave an area of illumination 0.35 mm. in diameter. The light reflected from a heliostat was directed by the mirror through this lens, by which it was concentrated on the object. By means of clamps under the fixed stage various absorptive screens—several kinds were used—for shutting out the dark heat rays and for producing lights of different colours could be fixed between the condensing lens and the mirror. These screens could also be placed above the fixed stage. For the object a small glass chamber was constructed, the floor of which was always covered with a drop of water—a most necessary precaution. Special metallic chambers, with glass floor and movable lid, were used for studying the effects of different gases. A dark screen for completely shutting off the light from the heliostat, and a neutral-tinted ocular, complete the special apparatus employed.

A considerable amount of heat is produced by the intense

lighting. This does not at first affect the tissue examined, immersed, as it is, in a drop of water, but as the degree of temperature gradually rises, and the drop begins to evaporate, the object may be acted on and eventually killed. In this way, then, there may be a heat effect from the temperature of the water in addition to that resulting from the illumination of the object. It is necessary to distinguish the thermal from the photochemical effects, although it is not always easy to do so, especially when the heating is very great, as it is in white and red light, and when the exposure is long and uninterrupted; and it may be necessary, in such cases, to resort to a cooling process in the course of experiment. This can be readily carried out by using a metallic slide, with a glass disk let in for the object and drop of water, and by inserting into the drop a small metallic ring with radiating arms. Ice laid on the slide or on these arms rapidly cools the water drop to the extent required.

It is important, then, to determine the temperature reached in the water drop during the short time—five to fifteen minutes—which suffices for the completion of the photochemical changes. This temperature naturally depends upon the colour of the intense light, upon the size of the drop and its original temperature, and upon the extent of cooling in the research.

Two methods were employed in these researches for the temperature determinations. (*a*) The insertion of a thermoelectric couple of iron and nickel into the drop, the results being read off by a galvanometer; and (*b*) the introduction of small crystals of substance of known melting point. For this latter purpose two substances, azoxybenzol, which melts at 45° C., and mint-camphor, with its melting point 35° C., were found most convenient.

The following are the determinations so made in this series of experiments:

In white light, the original temperature being 20° C., it rose in one to three minutes to 45° C.

In red light (fig. 28, spectra  $\gamma$  and  $\delta$ ), produced by a 5 mm. thick solution of iodine in bisulphide of carbon, allowing passage of red rays up to those of wave-lengths  $\cdot 00061$  mm., and also traces of violet rays of wave-lengths  $\cdot 00043$ — $\cdot 00041$  mm., a temperature 45° C. was attained in three to five minutes,—a most injurious one for plant tissues.

In both these lights the effects may thus be thermal as well as photochemical.

In green light (fig. 28, spectra  $\zeta$ ,  $\eta$ ,  $\theta$ ), produced by a 5—6 mm. thick copper chloride solution, allowing passage of

rays occupying middle of the solar spectrum, having wave-lengths between  $\cdot 00060$ — $\cdot 00045$  mm.; and in blue light (fig. 28, spectra  $\iota$ ,  $\kappa$ ), produced by a  $\frac{5}{8}$ —6 mm. thick solution of ammonia copper sulphate, giving passage to the strongly refractive rays of wave-lengths from  $\cdot 00051$  and  $\cdot 00053$  mm., after fifteen to twenty minutes' exposure the temperature rose, even when the original temperature in the drop was  $20^{\circ}$ — $25^{\circ}$  C., to only  $35^{\circ}$ — $36^{\circ}$  C.,—a degree in no way hurtful to plant cells.

Green and blue are thus the most favourable for investigations with intense light, as in them the effects are strictly photochemical.

### III. *Effect of Light. Paralysis and Death from Light.*

A. *Concerning the appearances produced in Plant Cells by the action of Intense Light.*—The time which elapses before visible effects appear in a cell exposed to intense light varies according to the specific energy of the cell. In most plants, and without exception in green plants, a few minutes suffice for their appearance, and the extent of the action can be regulated so that transformations may be brought about in the cell content without injury to the life of the cell, or paralysis and death of the cell may be produced. Some cells, however, especially non-green ones, for example, colourless swarm-spores which are very sensitive to heat, are but slightly affected and may remain for half-an hour or more exposed to concentrated white light without injury,—an important fact, as showing that a cell is not of necessity killed or injured when exposed in water to the heat which is generated in the focus of a large lens.

For investigation of the effect of intense light on green tissues, *Algæ*, *Characeæ*, moss leaves, hairs and soft leaf-tissues of *Fluviales*, are favourable subjects, as in them the effect is generally apparent in from two to six minutes.

If species of *Spirogyra*, such as *nana*, *jugalis*, *quinina*, or *Weberi*, be exposed to intense light, the effect immediately observed is complete disappearance of colour from the chlorophyll-bands. The ground-substance remains unchanged in form and disposition, and the marginal projections and amyllum-bodies persist (fig. 19 *a*, *b*, *c*, *d*). The band appears as if acted upon by alcohol, and the decolorisation is limited to the insolated area, though at times there may be a slight halo. It is possible to restrict the action of the light to one coil of the chlorophyll-band or to a portion of it, or, if a plant be employed with isolated



chlorophyll-corpuscles, some or a single corpuscle may be acted upon.

But, besides destroying the colouring matter, the light affects the cell-contents also, and especially those elements concerned in respiration and nutrition. In *Spirogyra*, which, in consequence of its organisation (with the nucleus embedded in a central plasma from which threads radiate to the periphery, and with a granular motion in the protoplasm between the chlorophyll-bands) is very suitable for study, either before or after complete decolorisation of the bands, an extending destruction of the cell-contents is observed. The motion of granules in the protoplasm of the cell-wall ceases. The threads of protoplasm contract and pull thereby the middle part of the coils of the chlorophyll-bands deep into the lumen of the cell, and at the same time a portion of their substance withdraws to the central plasma-mass, which swells into a large vesicle with a distinctly double-contoured membrane of considerable thickness (fig. 11 *b*). Then the threads rupture, and the proximal ends remain as fragments attached to the central plasma, whilst the distal portions adhere to the amyllum-bodies. Whilst these changes are progressing there appear upon the threads small, defined, vesicle-like formations (fig. 11 *a*), which are undoubtedly distinct morphological structures, and in the normal uninsolated cell are occasionally seen, as before mentioned, at the forks of the threads. These plasma-knots, as they may be called, which are also to be seen in *Spirogyra* cells killed by other means, are withdrawn with the substance of the thread to the central plasma, and coat its surface, often in great numbers (fig. 11 *b*). Through the contraction and rupture of the threads the nucleus is pushed from its central position and displaced to a greater or less extent (figs. 19), always surrounded by the central plasma with its coating of plasma-knots. It, along with the nucleoli, retains its form, but sometimes, at least in one-spined species of *Spirogyra*, appears to have lost portion of its substance, whilst in other cases it becomes granular in aspect and acquires a red tinge. The turgescence of the cell is markedly affected (in fig. 18, between *d* and *e*, the partition wall bulges into the insulated cell), and alterations in the substance of the layer of protoplasm lining the cell-wall, which are chiefly recognisable by colour changes, occur.

Many of these effects in the protoplasm are familiar as appearances connected with death of the cells from other causes, *e.g.* from heat or mechanical injury; but in such cases the changes are not identical in their whole course

with those just described. Here the effects are the result of a definite photochemical action, and many of them are specific of death from light, although they are combined with the general changes in the protoplasm, which appear after death from any cause.

Effects similar to those seen in *Spirogyra* occur, with modification dependent upon organisation, in other *Algæ* with so-called formless chlorophyll-masses; for example, in *Mesocarpus* (fig. 20), *Edogonium*, *Draparnaldia*, *Vaucheria*, &c., always varying in extent, up to complete death of the cell, according to the duration of exposure to light. Also in cells with definite chlorophyll-corpuscles, such as occur in *Characeæ*, *Musci*, ferns and phanerogams, like results obtain. Amongst these, *Characeæ* are most favourable for investigation on account of the power of recovery of their cells even after considerable injury, the structural relationships of these, and especially their great length, which permits of a small portion of one cell being insulated.

If a portion .3 mm. in diameter, of a *Nitella* cell, which is about 1 mm. long, and not too thick, be insulated, it is more or less completely decolorised in a few, usually three to eight, minutes (figs. 31, 32). Quite independently of the decolorisation, except in so far as the chlorophyll colouring matter acts as a screen, the destruction of the cell-contents begins and may extend until the whole content is killed. It is possible, however, to regulate the action so that death of the cell shall precede complete decolorisation of the insulated area, or shall succeed it, or that the only effect shall be the destruction of the colouring matter without injury to the life of the cell. This depends, in the first instance, upon the specific energy of the plant but also upon the depth and the size of the cell.

In deep plant cells, such as those of adult leaves, or the internodal cells of *Nitella*, which are lined with a dense persistent layer of chlorophyll-corpuscles, the depth of the cell and the protection of the layer of chlorophyll-corpuscles have great influence in modifying the action. The cell in such a case lies with its lower, or an ideal median, or its upper surface in the plane of most intense illumination, and the parts above or below such plane must be affected in a different degree. The layer of chlorophyll-corpuscles on the lower surface of the cell will protect in great measure the portions above so long as the chlorophyll colouring matter is not destroyed, and in like manner the centre of the layer of chlorophyll-corpuscles on the upper wall of the cell will be sooner decolorised than the sides of

of the cell where many rows of chlorophyll-corpuscles lie over one another. In *Spirogyra* like circumstances have to be considered, but only in small degree even in the species with deep cells, because of their small diameter, and also because the lower flexures of the bands afford but a small protection to the upper. Yet one finds that the points on the upper flexures where the lower flexures cross them especially at the edges of the cell-wall, and the spots where the bands commence to bend on the lower surface of the cell are later in being decolorised than the freely exposed and unprotected parts. A simple unscreened layer of chlorophyll-corpuscles, or a chlorophyll-plate, such as that of *Mesocarpus*, when it lies in a plane at right angles to the light, is decolorised in from one-and-a-half to two minutes. The layer on the upper wall of a *Nitella*-cell requires in light of equal intensity five to eight minutes of insolation.

But the length of the cell modifies the destruction of the cell-content when exposed to intense light. As the protoplasm in the cells of *Nitella* is in constant slow streaming movement during examination, any one portion of the protoplasm is only subjected for a relatively short period to direct insolation, dependent, indeed, upon the size of the insolated area as compared with the size of the whole cell, and thus the protoplasm is acted upon by the light interruptedly. Each portion of the protoplasm, as it rotates round the sides of the non-illuminated part is protected from light, and only again is acted on when it reaches the insolated area.

In *Nitella* the destruction of the chlorophyll colouring matter depends therefore mainly upon the depth of the cells, whilst the destruction of the protoplasm is also influenced by the length of the cell; so that the immediate appearances in the local destruction of a *Nitella* cell may be very different.

If the cell be a long and strongly grown one the complete decolorisation of the insolated part may occur without any trace of further destruction of content. The chlorophyll-corpuscles in the non-illuminated part remain normal in form, colour, and disposition. The neutral zone persists, and there is no retraction of the protoplasmic utricle from the cell-wall. Rotation of the protoplasm and contained bodies shows, as a rule, no visible disturbance; there may, however, occasionally be a momentary cessation of the movement.

In other cells, and specially if the insolation of a limited area be rapid, death throughout the whole cell may occur long before complete decolorisation of the insolated part, or even before the chlorophyll-corpuscles in this position show much

trace of it. The first striking change in such a case is stagnation of rotation, with which is associated an irregular aggregation of the protoplasm at the insulated area which hinders the movement. Upon this follows an extending destruction of the cell-content. The green chlorophyll-corpuscles throughout the non-illuminated portion fall into disorder, lose their arrangement in rows (fig. 24), and swell in a manner commonly seen in them when they escape from the cell; they change their polyhedral or oval form and become, by the absorption of water, transformed into vesicles such as Göppert and Cohn describe,<sup>1</sup> and their contents, hitherto hardly recognisable, now appear sharply circumscribed. Finally, the protoplasmic utricle separates from the cell-wall, and all the signs of death from light are apparent. In the insulated part the chlorophyll-corpuscles exhibit none of these changes, nor does the protoplasmic utricle separate from the cell-wall; and this portion may remain in this condition for months, long after the adjacent chlorophyll-corpuscles have disintegrated.

If before death is set in and before the chlorophyll-corpuscles are completely decolorised the light be interrupted, then the partly or almost entirely decolorised chlorophyll-corpuscles separate from the utricle and fall into the rotating protoplasm, and with the formed elements circulate uninterruptedly in its current, forming often small heaps of corpuscles without disturbing the rotation, and though they gradually lose colouring matter, and after a time are quite colourless, they suffer no further change in form or substance. By degrees in this way the insulated part becomes deprived of chlorophyll-corpuscles and may get quite bare of them, and *Nitella*-cells in this condition may be kept for months unchanged, the rotation continuing vigorous, and the formed elements in the protoplasm and the chlorophyll-corpuscles in the non-illuminated area retaining their normal form, colour, and arrangement (fig. 23).

In other cases, again, if the action of light is of a high intensity, and the insulated area is decolorised without the rotation being stopped, there are often developed, especially in long cells and when the insulated area is in the middle of the cell, two currents instead of one, which rotate at opposite ends of the cell, each independent of the other, and separated from one another by the insulated area, which is a barrier to the movement.

But this action of light is not confined to green tissues. Non-green cells—for example, the blue-sapped hair-cells on the stamen of *Tradescantia virginica*—are affected in the

<sup>1</sup> 'Bot. Zeit.,' 1849, p. 681.



same way, and paralysis and death may be produced (fig. 27). Paralysis may occur before or after destruction of the blue colouring matter of the cell-sap, and the circulation, which is temporarily suspended, may after a longer or shorter time return without the cell having suffered at all. Death occurs in them also before complete destruction of the colouring matter. Whenever the colour changes to violet the protoplasm is killed, circulation is stopped and never returns, and the protoplasm threads become granular, in part disappear or rupture irregularly, and the protoplasmic utricle, separating from the cell-wall, collapses. A slight separation of the cuticle over the insulated area is sometimes seen.

B. *Conditions of Light Action in Cells.*—The effects just described are dependent not only on the high intensity of the light, but also upon its colour and the affinity for oxygen of the cell-contents. All of them are produced rapidly and energetically in white light. In coloured light, on the other hand, there is not only a marked retardation in rapidity of their appearance, but in red the light effect is suppressed, although in dark green and blue the changes are completed in a few minutes. The decolorisation and death of the cells, which in white light are brought about in two or three minutes, are accomplished in about five minutes in green and blue light (Section vii, Exp. 1st Series). All these researches show that in any yellow, green, or blue light it is easy to decolorise and kill the cells of many *Algae*, *Characeæ*, *Musci*, *Filices*, and *Phanerogamæ*, provided the colour employed is not too dark; whilst in red light of the same intensity, and after twice or four times as long exposure, no changes take place, and the red rays therefore appear to be protochemically inactive, or at least only very slightly active, on plant-cells.

That these effects are not due to the heat, considerable as it must be in some coloured lights, generated at the focal point of the large lens used, is evident from a comparison of the changes accompanying death in cells from heat with those here described. The destruction of the colouring matter will not be confounded in the two cases, but the changes in the protoplasm, the paralysis and actual death of the cells are alike, though not identical, from both causes. This subject requires, therefore, further notice.

In the first place, then, the temperature of the drop of water in which the plants are examined does not rise sufficiently high to cause the destructive action in the cells. That this is the case may be assumed from the slow evapo-

ration of the water in the drop, by the presence of many animal and vegetable organisms alongside of the specimen examined—Flagellata, Radiolaria, &c., moving quietly about, often in the illuminated area, without suffering any harm—and, lastly, by the exemption from the action of the cells adjacent to those exposed. Moreover, in blue and green light the temperature never can reach any high degree (see pp. 87 and 88).

Within the cells themselves, too, where action of light is manifested, no purely heat effect, such as might be produced by invisible heat rays, occurs. In conformity with our knowledge of heat distribution in the spectrum, and of diathermancy of elementary gases and liquids, the temperature determinations already given (pp. 87 and 88) show that when the light passes through a solution of iodine in bisulphide of carbon (spectra  $\gamma$  or  $\delta$ , fig. 28), much more heat is developed than when under like conditions the light passes through a solution of copper chloride and ammonia copper sulphate (spectra  $\zeta$ ,  $\eta$ ,  $\theta$ ,  $\iota$ ,  $\kappa$ , fig. 28). In any red light the temperature in the water drop is raised within five minutes to over  $45^{\circ}$  C., and yet after fifteen to twenty minutes of exposure there is neither decolorisation nor death of the cell. On the other hand, in green and blue light, cells are decolorised and killed in five minutes, although after fifteen to twenty minutes uninterrupted exposure the temperature scarcely reaches  $35^{\circ}$ — $36^{\circ}$  C.—a degree quite harmless to the cells. Now, although these temperatures in the drop and the illuminated area are not an exact measure of the temperature reached within the cells themselves, and it may therefore hardly be admitted that in the case of the green and blue light the changes are evoked by a temperature, at the highest, of  $35^{\circ}$ , yet the circumstance that the warm red rays have no effect, whilst the cold green and blue ones are active, shows that in producing these changes in the cell the intensity of light effect of the rays is not proportional to their heating power. The destruction in the protoplasm which leads to the death of the cell depends, therefore, not alone upon the thermal activity of the rays, but is to be referred rather to their chemical activity, in which colour is of great significance. How far the light affects the chlorophyll colouring matter alone is subsequently discussed, but in passing it may be noted that the inert red rays are more strongly absorbed in the chlorophyll colouring matter (compare spectra  $\beta$  with  $\gamma$  and  $\delta$ ) than the active green.

The relation of the action of light to the chemical processes in plant cells is made more clear when the plants exposed to the intense light are placed in different gases or

gas-mixtures. If *Spirogyra* or *Nitella* cells are exposed to light of any colour, and the ordinary atmosphere of the gas chamber is replaced by one of hydrogen free from oxygen, or of a mixture of hydrogen and carbonic acid, they show after twenty minutes no change of colour or of their normal character, and may be kept in this condition for weeks provided other detrimental agencies are excluded. Indeed, green and non-green cells which, in presence of oxygen, even in the relatively cold green and blue light, are killed after an exposure of three to five minutes, remain uninjured, when oxygen is excluded in light of any colour so long as the exposure is not so protracted as to allow of development of hurtful heat effects (see Section vii, Exp. 2nd Series). The temperature obtaining in such cases in the cell-contents, in each chlorophyll-corpusele and in each plasma-molecule, high as it must be, cannot then be the essential cause of the appearances produced. The substitution of hydrogen for the ordinary air is accompanied by no reducing action causing warming of the object. The light and heat absorption of the chlorophyll-corpuseles and the other constituents of the protoplasm remain unchanged; and heat-effects and conduction are essentially alike in both atmospheres. If, therefore, the destruction of the cell-contents takes place in an atmosphere containing oxygen, but is in abeyance when no oxygen is present, those molecular changes, through which dark heat-rays kill the cell at a lower temperature, do not proceed in the protoplasm. The correct explanation of the appearances seems to be that the light influences the relation of the plant to the oxygen of the atmosphere, and that the illuminating rays increase the chemical affinity of the cell-contents for oxygen. The amount of this action is dependent on the colour of the illuminating rays, and increases with their refraction from the red to the blue end of the spectrum.

The red rays have been shown to have no distinct photochemical effect. This is true, however, only for rays of a very definite position in the spectrum, from the extreme red to those of a wave length of  $\cdot 00061$  mm. (spectrum  $\delta$ , fig. 28). But even under these, after prolonged exposure, or if the cells are very sensitive, small effects are visible, though it may be doubted whether all are purely chemical and some are not thermal. Thus, the sensitive cells of *Mesocarpus scalaris*, if exposed to red light (spectrum  $\delta$ , in which, however, are traces of violet), are killed in two to three minutes, but the chlorophyll remains uninjured (fig. 21). Now, as in this spectrum, the temperature rises to  $45^{\circ}$  C.

in a few minutes, the cells may be killed by heat, for in other cases, *e.g.* in *Spirogyra*, after ten to twenty minutes exposure the same spectrum is not entirely without effect. But it is also possible to regard the death here as the result of photochemical action on the protoplasm before the chlorophyll colouring matter is destroyed; and this opens the question, presently to be dealt with, of the relative sensibility of the several cell-elements.

The absolute non-activity of the red rays is, therefore, not maintained, but what is established is this, that the amount of the oxidising action of light on the plant-cells sinks in a very striking manner towards the red end of the spectrum. The increase in activity towards the blue end has not as yet been quantitatively estimated, and a curve, expressive of the relative amount of respiration in the cells for the different rays of the spectrum, cannot therefore be drawn. Indeed, the varying intensity of illumination which must occur in the concentration of light upon dense masses, the great difference in intensity produced by slight deviations of the object from the plane of intense illumination, and the want of a standard for finding the amount of action within the cells before they are killed by the light, make such a quantitative determination almost impossible. All that can be said from a consideration of the light-absorption in the chlorophyll colouring matter is, that generally a rise, and a decided rise, of effect takes place in the blue.

But careful consideration of the intensity of the active colour is necessary, for it is found that in high intensities the cells are killed more rapidly in yellow and green. A dark and light solution of copper chloride, which differ little in breadth of their absorption-spectra, show a great difference in their effect upon the plant-cells. Thus, light with the spectrum  $\zeta$  (fig. 24), produced by transmission through a 10 mm. thick layer of copper chloride solution, acts very slightly; one with spectrum  $\eta$  acts more strongly; whilst one with spectrum  $\theta$  produced by a 5 mm. thick layer of the same solution, and which has a trace of blue, causes a much stronger light-effect than either of the others. Blue light produced by a solution of ammonia copper sulphate, although this, as was mentioned before, absorbs blue rays to a great extent, and therefore in a concentrated condition allows but a feeble blue light to pass, acts equally strongly. If a comparison is to be made between the light action of blue and green rays so produced, the blue-light solution must not be too dark, for this has led to many errors concerning the energy of colour in assimilation; and it must also be remembered that the light solu-



tions of ammonia copper sulphate allow a little of the dark green to pass, and brighter copper chloride ones give passage to even more blue. The feebly illuminating blue rays appear then more active than the more strongly lighting green; and this throws an unexpected light on the significance of green colour to vegetation, for the blue rays which are most active in producing oxidation of the cell-contents are almost completely absorbed.

*c. Combustible and Incombustible Constituents of the Cell in Intense Light.*—The destructive action of intense light in plant-cells proceeds only in presence of oxygen. Some only of their constituents are oxidised, others, even in the most intense light, are incombustible. One is justified in assuming that amongst the substances in the cells which, by their behaviour in intense light, show great affinity for oxygen, are to be found the special combustible elements of normal respiration in the green tissues of plants, and that those contents which remain unchanged in presence of oxygen in intense light, can in no way under normal light-conditions serve as supporters of respiration. The behaviour in intense light of the larger and well-known formed constituents of cell-contents is easily made out, but more observations are required to determine which among the more minute yet definitely-formed bodies in the protoplasm are stable and which are unstable.

1. *The colouring matter of cells in intense light.*—The changes which the colouring matter undergoes are easily witnessed. As has already been pointed out, the colour vanishes from green cells in a few minutes when exposed in presence of oxygen to intense light, though this does not occur when the light is red. No new substance is found in the cell as a product of this destruction, nor is there increase of the pre-existing constituents. It may therefore be conjectured that the colouring matter passes over directly into gaseous product of respiration. The carbonic acid present is not implicated in the destruction of the colouring matter, for in atmospheric air deprived of carbonic acid the decolorising process proceeds as quickly and energetically as in ordinary atmospheric air containing carbonic acid, whilst in a mixture of carbonic acid and hydrogen all photochemical effects are in abeyance (see Section vii, Experiments 28—31 and 37—40). It would appear, therefore, that green cells placed in a mixture (varying quantitatively) of carbonic acid and hydrogen, free from oxygen gas (in which assimilation is quite possible), and exposed to the influence of

intense light (which in other conditions kills and decolorises them), remain, notwithstanding the carbonic acid present, unchanged and green. This fact, that carbonic acid plays no part in the destruction of the green colouring matter, is of considerable importance in view of the theory of the function of chlorophyll colouring matter here advanced.

The cells once decolorised, whether to a great or slight extent, never, even though they be not killed, regenerate the chlorophyll colouring matter. The decolorisation sets in relatively slowly, becoming manifest only after a minute or so of exposure. The case of *Nitella*, as previously described, is a very typical one.

It may, then, be concluded, from such facts, that the destruction in nature of chlorophyll is a pathological process, hurtful to the plant, and not, as has been supposed, a normal process associated with the breaking up of carbonic acid, and the fixing of carbon in the green tissues.

Ever since the days of Senebier opinions have widely differed upon the destruction of chlorophyll in light, upon the relative energy in the process of the different rays of the spectrum, and upon the significance of the process in the life of the plant. Some have tried to settle the question by researches into the changes exhibited by a solution of chlorophyll when exposed to light—a method of no value without further observations upon the behaviour of chlorophyll when attached to the living plant. Others have made observations upon the plants themselves in their natural situations, studying their blanching, yellowing, and the changing in colour of parts exposed, especially in winter, to direct sunlight. But in such cases the conditions are complicated, and, moreover, the actual methods employed have been faulty. Tissues (especially of land plants) have been employed, after maceration for days or weeks in water, which naturally causes disease or death, wherewith are associated changes of colour, blanching, and destruction of chlorophyll-corpuscles; so that, in all such researches it has been doubtful whether decolorisation was the direct effect of light or the secondary result of some other injurious cause. Hence it has happened that the decolorisation is ascribed by one to frost, by another to heat, and by others to light.<sup>1</sup>

<sup>1</sup> For more detailed information regarding the destruction of chlorophyll in living plants consult:

Mohl, "Ueber die winterliche Färbung der Blätter," 'Verm. Schrift,' 1837, xix.

Sachs, "Abwechselndes Erbleichen und Dunkelwerden der Blätter bei wechselnder Beleuchtung," 'Berichte d. Sächs. Gesellsch. d. Wiss.,'

The only accurate method is to study the changes in the plant-cells, and, as a result of the investigations here recorded, the possibility of the destruction of chlorophyll colouring matter by intense light in the living cells is proved. Whether this goes on as a normal process is discussed at a later stage of this paper.

Not only chlorophyll colouring matter, but also yellow, blue, and red, are in presence of oxygen destroyed within the plant cells when exposed to intense light. Yet all colouring matter found in plant-cells is not so acted on; different colouring matters under like conditions behave differently. Observations are still wanted to show which are destructible and which are indestructible, and to supply the key to the relation between destructibility of these colouring matters of the cells in light and their genetic connection with chlorophyll colouring matter. As illustration: the red colouring matter of many resting spores of *Algæ*, especially oospores, is not destructible, or only with extreme difficulty, by exposure to intense light. On the other hand, the steel-blue colouring matter of the *Phycochromaceæ* (*Oscillariæ*, *Nostocaceæ*, &c), the brown of *Diatomaceæ*, *Phæosporeæ*, and *Fucaceæ*, and the red of *Floridææ*, in all their modifications, are, it appears, as easily destroyed in intense light as the chlorophyll colouring matter of green plants.

Colouring matter of flowers seems to behave differently. The yellow-red colouring matter of the ligulate flowers of *Calendula* is with ease completely destroyed in intense light, whilst that of *Narcissus poeticus*, in the same circumstances, remains unchanged. The blue cell-sap of *Tradescantia virginica* loses its colouring matter easily (fig. 27), whilst other blue flowers are decolorised with difficulty, or not at all (see Section vii, Experiment 48).

Leipzig, 1859; 'Flora,' 1862, p. 219; 'Handbuch der Experimentalphysiologie,' 1865, p. 15.

Askenasy, "Zur Kenntniss des Chlorophylls," 'Bot. Zeit.,' 1867, p. 228; and "Ueber Zerstörung des Chlorophylls lebender Pflanzen durch Licht," 'Bot. Zeit.,' 1875, p. 457.

Kraus, "Ueber winterliche Verfärbung immergrüner Gewächse," 'Bot. Zeit.,' 1872, p. 109.

Batalin, "Ueber Zerstörung des Chlorophylls in lebenden Organen," 'Bot. Zeit.,' 1874, p. 433.

Wiesner, "Ueber die Beziehung des Lichtes zum Chlorophyll," 'Sitzungsber. d. Wien. Akad.,' 1874; and 'Die natürlichen Einrichtungen zum Schutz des Chlorophylls,' Wien, 1876.

Haberlandt, "Ueber die Winterfärbung ausdauernder Blätter," 'Sitzungsber. der Wien Akad.,' 1876.

Böhm, "Ueber Verfärbung grüner Blätter im intensiven Licht," 'Landw. Vers. Stationen,' 1877, p. 463.

The subject is one demanding investigation, and further communications will be given.

2. *The ground-substance of the chlorophyll-corpuscles and its included substances in intense light.*—The chlorophyll-corpuscles serve not only as organs of assimilation, as has hitherto been supposed, but also as organs of respiration. This, their double function in gas interchange, is evidenced by the morphological and microchemical changes which their ground-substance and contained-bodies undergo in intense light. Though green organs under the influence of light give off oxygen, yet the chlorophyll-corpuscles, by reason of their structural and chemical characters, fix oxygen in a high degree and at once transfer it to the forming products of assimilation. The physiological value of these substances, formed and deposited within the chlorophyll-corpuscles, falls therefore to be considered after the behaviour in oxygen and in light of the bodies themselves.

The ground-substance of the chlorophyll-corpuscles and masses after insolation resembles in appearance the condition in which it is left after alcohol or other solvent has decolorised and removed the oil from it. It is a colourless skeleton, unchanged in form, and presents the same sponge-like structure already described as resulting from the action of solvents, acids, and moist warmth (figs. 1, 3, and 26). In *Spirogyra* the marginal projections on the band (so changeable in the living cell) remain; only sometimes when an excess of heat has operated, or a considerable time has elapsed before the death of the band, are the projections withdrawn and the band contracted in width, or the margins, still marked by the projections, become revolute. Chemically little can be said. The skeleton shows a protoplasmic character, and takes up iodine and colouring agents more readily than before, possibly on account of its porosity. Insolation, however, produces a change in the nature of the substance, in virtue of which it offers greater resistance to external influences. Such a change is well illustrated by the case already described of *Nitella*-cells killed by rapid insolation of a limited area, in which the decolorised chlorophyll-corpuscles of the insolated area remain unchanged in form and shape long after the green corpuscles and contents of the non-illuminated portion of the cell are completely disintegrated (fig. 24). The chlorophyll-bands of *Spirogyra* and the chlorophyll-masses of other plant-cells, which, as is known, are extremely sensitive to injurious influences, such as increase of temperature, mechanical irritation, &c., losing their form, contracting, rupturing, or swelling into variously-



shaped bodies, behave when decolorised by insolation in the same way as the chlorophyll-corpuscles in *Nitella*. The light-killed bands stiffen while retaining their normal form and configuration (fig. 19). The ground-substance of chlorophyll-corpuscles and masses appears thus under the influence of light to have lost its power of swelling in water and watery solutions, and in this, as in the retention of its form and shape, we have a marked difference between the destructive effects of light and of heat.

Before studying the effect of light on the bodies contained in the chlorophyll-corpuscles, it is necessary to consider what these substances are and how they are distributed.

From the time when Mohl first demonstrated the common association of starch with chlorophyll, and subsequently Böhm, by his improved iodine test, afforded means of more easily determining its presence in chlorophyll-corpuscles, starch has been regarded as a necessary construction product of chlorophyll-corpuscles. Mohl,<sup>1</sup> indeed, failed to discover it in many plants, and Böhm,<sup>2</sup> although he found it in many cases where Mohl had a negative result, yet mentions certain plants as possessing no starch, *e.g.* *Allium fistulosum*, *Asphodelus luteus*, *Orchis militaris*, *Lactuca sativa*. Some years later colourless bodies not consisting of starch were observed in chlorophyll-corpuscles, and these have usually been regarded as fat-globules or oil-drops. Thus Pringsheim,<sup>3</sup> in 1855, showed that in *Vancheria sessilis* there is no starch, but that small oil-globules occur which supply the formative material for the oogonia. Nägeli<sup>4</sup> afterwards pointed out their existence along with or instead of starch in the chlorophyll-corpuscles of *Rhipsalis funalis*, *Cereus variabilis*, and in non-green freshwater and marine *Alge*. Then Sachs<sup>5</sup> showed that there is no starch in *Allium cepa*; and finally, Briosi<sup>6</sup> noticed that starch is wanting in the chlorophyll-corpuscles of *Musacca*, but that

<sup>1</sup> "Untersuchungen über die anatomischen Verhältnisse des Chlorophylls," Dissertation vom Jahre, 1837 ('Verm. Schrift,' xxvi); and "Ueber den Bau des Chlorophylls," 'Bot. Zeit.,' 1855. In his paper of 1837 Mohl recognised that the starch content of chlorophyll-corpuscles was a reserve material for the plant.

<sup>2</sup> "Beiträge zur Kenntniss des Chlorophylls," 'Sitzungsber. d. Wien. Akad.,' October, 1856.

<sup>3</sup> 'Monatsber. d. Berl. Akad. d. Wiss.,' March, 1855.

<sup>4</sup> 'Die Stärkekörner,' p. 398; 'Pflanzenphysiologische Untersuchungen,' Pt. 2, 1858.

<sup>5</sup> 'Bot. Zeit.,' 1862, p. 369; 'Flora,' 1863; 'Experimental Physiologie,' p. 28.

<sup>6</sup> 'Bot. Zeit.,' 1873, p. 529. Briosi's results, as Vines points out in 'Nature,' xxiii, p. 562, have been shown to be incorrect by Holle and Godlewski in 'Flora,' 1877.

a normal formation of oil-globules proceeds in them, and he concluded that they must regularly pass out of the same. But besides those mentioned, many other plants are known in which no starch is found and oil-globules occur in the chlorophyll-corpuscles. Thus, of plants without starch we have *Selaginella*, *Cycas*, *Stratiotes aloides*, *Lilium Martagon*, *Olea europæa*, and *Begonia*. The small refractive oil globules of the bands of *Spirogyra* have been long known, and in *Characeæ* the chlorophyll-corpuscles possess oil globules in addition to or instead of starch, which pass out of them in the course of development.

Starch and oil have, from their easy recognition, been often hitherto regarded as the only products of the function of the chlorophyll-corpuscles. In some cases, when neither starch nor oil has been found, and abundance of glucose or mannite has been discovered in the leaves of a plant, these substances have been considered as the product of the chlorophyll-corpuscles. But neither starch, oil, nor sugar are the only products, nor, indeed, the only visible ones. Pringsheim in one case observed highly oxidised bodies of the group of organic acids directly formed in the chlorophyll-corpuscles, and this makes it probable that of the bodies hitherto considered to be fat or oil-drops many consist of substances of a very different character. Again, in *Mesocarpus scalaris*,<sup>1</sup> at all stages of development and in every cell, there may be seen numerous (almost covering the chlorophyll-plate) small, oil-like, glistening globules of different sizes, and very like the small oil-globules in the bands of *Spirogyra*, which, as they disappear on the addition of alcohol and ether, might at first be regarded as fat or oil-globules. They are clearly formed in and secreted by the chlorophyll-plate, and pass out from it into the protoplasm of the cell. These are not oil-globules but vesicles with a resisting pellicle, enclosing a content in greater or less part consisting of tannin. They may be termed tannin-vesicles (fig. 20 c). The dark coloration and coloured precipitate formed with iron salts, potassium bichromate, and with Millon's reagent, conclusively indicate their nature (fig. 22). The form of these vesicles is easily destroyed by slight warmth, mechanical irritation, or any decomposition which will end in the death of the cell, and when destroyed the contents disappear, mixing with the cell-sap, and the pellicle is then indistinctly seen appressed to the chlorophyll-plate, and giving its surface a netted or froth-like aspect (fig. 21).

<sup>3</sup> This species was determined from specimens just commencing to conjugate. It is the form generally known as *Mougeotia gemiflexa*.

In addition to all these substances the oily vehicle of the chlorophyll colouring matter and the hypochlorin must be reckoned as contained elements of the chlorophyll-corpuscles and as normal products of their function.

The different elements and constituents of the chlorophyll-corpuscles behave differently when exposed to intense light. Some are affected, others are not, and it is the merit of the method of investigation now being elucidated that it enables a direct study to be made of the relations of these constituents to respiration.

Of the constituents just mentioned, those which are rich in oxygen and occur as grains, globules, or vesicles—the starch-grains, the oil-globules, the tannin-vesicles—are unaffected by exposure to intense light.

Starch-grains in the chlorophyll-corpuscles have the same appearance, and exhibit the same reaction with iodine, after, as before insolation, and the same is true of the starch in the amylum-bodies of *Spirogyra*. With the destruction of the chlorophyll colouring matter no formation of starch is associated. If starch was present in the chlorophyll-corpuscle before insolation, it is found in like amount afterwards; if there was none before insolation, none is found afterwards. There is neither destruction nor formation of starch as the result of exposure to intense light. This is no contradiction to the well-known fact of the accumulation of starch in the chlorophyll-corpuscles during the day. For the explanation of that is very simple. Starch is not directly formed from the decomposition of carbonic acid and water; and, if in light of no great intensity there is an accumulation of starch in the chlorophyll-corpuscles, it happens because the light was not sufficiently intense to destroy the formative material out of which the starch is constructed.

Oil globules, both within and outside the chlorophyll-corpuscles, behave in every respect like starch. At least all the colourless and oil-like globules, such as occur in the bands of *Spirogyra*, in the chlorophyll-corpuscles of *Characeæ*, *Vaucheria*, *Fontinalis*, &c., which are to be considered as of a fatty and not of a volatile oil, do.

Tannin-vesicles of *Mesocarpus* are indestructible by light alone. They are, as already noticed, very sensitive to hurtful agencies, and when the cell is killed by light they are, of course, decomposed; but this is rather a secondary effect, the result of the death of the cell, which has been already brought about by light.<sup>1</sup>

<sup>1</sup> These tannin-vesicles in *Mesocarpus* are instructive in showing the varying effect of light of different colours. Thus, in red light the tannin-

Hypochlorin, on the other hand, disappears from cells exposed to intense light, and no trace of it remains. If a filament of *Edogonium*, *Spirogyra*, *Cladophora*, or *Mesocarpus* be exposed to intense light, there is decolorisation of the insulated area; the cells of the rest of the filament are unchanged. If now dilute hydrochloric acid be added hypochlorin appears in the usual way, after from six to twenty four hours, in the non-exposed areas, but none is seen over the insulated portion. If, in *Spirogyra*, insolation is interrupted before the chlorophyll colouring matter of the bands is dissipated, no hypochlorin is found on the slightly or half-decolorised bands, though it is present on the non-insolated bands. Five to six minutes of insolation suffice to decolorise the bands of *Spirogyra jugalis*; under the same conditions two to three minutes are enough for the destruction of the hypochlorin. Where there are isolated chlorophyll-corpuscles the same is observed, *e.g.* if a portion of a cell of *Nitella* be exposed (fig. 25). In this plant, however, it sometimes happens that no hypochlorin shows on the green chlorophyll-corpuscles of the non-insolated portion of the cell. The light effect in this case, it would appear, has spread beyond the immediately exposed area, and this may be explained by the fact that hypochlorin is one of the most easily affected bodies in the cell. Small increments of temperature, mechanical stimuli, spontaneous disease, as already shown are, even when the chlorophyll colouring matter is intact, able to destroy it in the cell. From these facts we may conclude that the disappearance of hypochlorin is the earliest indication of a hurtful influence affecting the plant cell, and its destruction results in intense light earlier than does the destruction of the chlorophyll colouring matter.

3. *Protoplasm of the cell and turgescence as affected by intense light.*—The turgescence of cells is diminished by exposure to intense light. In large-celled *Algæ* this is shown by the vertical division wall between insulated and non-insolated cells becoming curved into the former (fig. 18, between *d* and *e*). The tension of the insulated cell is decreased relatively to the non-insolated by the greater permeability (as shown by its behaviour to coloured solutions) for cell-sap of its protoplasmic utricle. If a filament of *Spirogyra*, some cells of which have been insulated, be laid in a watery solution of vesicles are at first broken, the cell contents collapse, and the chlorophyll-plate contracts, but the colour of the plate remains unchanged (fig. 21). In blue light in the same or in shorter time the chlorophyll-plate loses its colour and contracts, the cell contents collapse, but the tannin-vesicles remain for some time intact, and only at a later period coalesce when the insolation has entirely ceased (fig. 20).



aniline-blue, the insolated cells become rapidly coloured, the non-insolated are, even after some days, still uncoloured. *Nitella* is very favourable for such an observation, as the differences, owing to the length of the cells, may be observed in one and the same cell, and moreover, as the rotation is not stopped, one may study the difference in permeability between the cell-wall and the protoplasm, the former becoming rapidly coloured, whilst beneath it the rotating protoplasm remains for a time unchanged.

The greater permeability after insolation is associated with a distinct change in structure and in mass of the protoplasm lining the cell-wall. It loses, as has been shown, a considerable amount of its contractile power. The addition of plasmolytic agents, such as iodine in iodide of potassium, causes little or no retraction of the utricle in an insolated cell (fig. 19), and it depends upon the intensity and duration of exposure to light, as well as upon the thickness of the layer of protoplasm, to what extent the power of contraction is lost. In *Nitella* this may be well studied. Here, as has been already described, the contraction consequent upon death from insolation advances gradually over those portions of the cell which have not been insolated and are green, the protoplasm at first slowly separating from the cell-wall, and then subsequently collapsing. Only at the insolated part does the utricle show no, or almost no, contraction, and may be found with embedded skeletons of chlorophyll-corpuscles still lining the cell-wall months after insolation (fig. 24). The reason for this is a partial destruction of the protoplasm, which is the immediate effect of the light, and as light only acts in presence of oxygen it is clear that the oxygen must combine with certain elements of the protoplasm of the utricle, and, as a result of the combustion, that change and diminution of its substance occurs which produces a loss of contractility.

This loss of substance by oxidation in light may at times be made directly visible. If a filament of *Spirogyra* (one-spined species are most suitable, as in them the utricle is not strongly developed), some cells of which have been insolated, be treated with a reagent which stains protoplasm deeply (iodine solutions, for example), a more or less striking difference is observed between the contracted protoplasm of the insolated and the non-insolated cells. In the non-insolated cells the former lining protoplasm of the wall no longer forms a continuous uniform layer or plasma-utricle,<sup>1</sup> but

<sup>1</sup> Demonstrated by Pringsheim in 1854, in 'Untersuchungen über den Bau und die Bildung der Pflanzenzelle,' p. 10.

is a contracted plasmodium-net, in which are embedded, as in a matrix, small dense granular or globular bodies. These, especially, are deeply stained by the iodine (fig. 18). If the protoplasm lining the wall was originally thick they are numerous; if it were thin they are few in number, sparingly distributed, and may be almost absent. But their number depends on the species examined, as well as upon the thickness of the utricle. In the insolated cells one can recognise with certainty a diminution in the number of these small bodies. In them is doubtless to be recognised the element that so readily takes up oxygen under the influence of light, and by its combustion causes the loss of substance in the protoplasm, whereon depends the diminution in contracting power. It is often difficult to determine the loss of protoplasmic substance, and it can only be done by careful comparison of insolated and non-insolated cells.

Other striking changes after insolation in the protoplasm have already been referred to as occurring in *Spirogyra* and in *Nitella*. In the former there is the displacement of the nucleus, vesicular swelling of the central plasma concurrently with a granular coagulation of its substance and the occasional colouring of the same, and finally, the rupture and knotting of the protoplasm threads. In the latter there is the aggregation of the streaming protoplasm at the area of insolation, &c. Many of these changes doubtless occur in general death of protoplasm from other causes besides light, for example, from heat or electricity, and it is therefore difficult, if not impossible, to distinguish amongst them the specific action of light.

Amongst the phenomena which fall to be noticed here is paralysis of protoplasm, or temporary stoppage of its movement by light. If insolation be interrupted just at the moment when the motion ceases, in many cases the movement will sooner or later recommence and go on normally. This may be observed in all kinds of movements, alike in the motion of granules in the protoplasm between the chlorophyll-bands of *Spirogyra*, in the circulation in *Tradescantia* hair-cells, and in the rotation in *Nitella*. The condition of paralysis in the cells of staminal hairs of *Tradescantia virginica*, and in suitable (short) cells of *Nitella*, may develop long before they are decolorised. Careful study of the gradual cessation of movement in intense light convinces one that the light at the place where it reaches the protoplasm creates directly a hindrance to the movement, a fact explicable by the changes (just described)

it brings about in the substance of the protoplasm. As soon as light has operated sufficiently long, one sees, *e.g.* in *Nitella*, the movement slowing at the insulated spot, and the streaming protoplasm in consequence aggregates in large, often striated, masses about it. Then, especially if the cell be long and the insulated area near its middle, as the current cannot cross the insulated area, two currents frequently arise in one cell, each following a course of its own, separated from the other by the insulated part.

These phenomena are to be explained by the greater immobility of the protoplasm which has been exposed to light. Because protoplasm aggregates at the insulated area, it does not necessarily follow that it is as it were drawn by the light, but only that in certain intensities the motion of the protoplasm is slowed at the insulated area. And so also the opposite effect, the aggregation of the protoplasm at the shaded spots, is to be explained by the commencing immobility of the insulated portion forcing the protoplasm into other channels. The disposition of the moving masses in the cell shows only the relative motility of the protoplasm in different, relatively darker and lighter, places corresponding with the oxidation of its substance as influenced by light.

4. *The membrane of the cell in intense light.*—The cell-wall exhibits no very striking changes. At times, in more delicate *Spirogyra*-cells a slight swelling may be seen. If, however, delicate species of *Spirogyra*, *e.g.* *Sp. Weberi* or species of *Mesocarpus*, be exposed to light until death of the cells sets in, the insulated cells separate more or less completely one from another (figs. 20, 21), and in *Sp. Weberi* the infolded ends of the cells unfold. The changes in turgescence of the insulated cells appear to have nothing to do with this appearance; nor is any perceptible shortening or lengthening of the cell walls apparent. But at the moment of separation a slight torsion of the threads is observable, and possibly an inequality in tension between the cuticle and the inner layers of the membrane, developing under the influence of insolation, may cause the torsion and consequent separation. It is possible, however, that chemical changes of the membrane at the limits of the cells may contribute to the result.

#### IV. *Respiration in Light of Green Tissues and the function of Chlorophyll.*

All the forementioned results lead to the conclusion that the amount of oxygen inhalation exercises a very definite influence upon the assimilation by chlorophyll-corpuscles,

and hence the green colour is of physiological value to assimilating organs, even if it play no direct part in the decomposition of the carbonic acid. The illuminating light-rays exercise an immediately-observable effect upon the colourless protoplasm of the cells, especially upon the ground substance of the chlorophyll-corpuscles and on their enclosed substances. The destructive action within cells in intense light, when oxygen is present, and the immunity observed in its absence, indicate that the injurious effect of light is due to increased combustion of the cell-elements necessary to life. It is still a moot-point whether the cell-elements which absorb oxygen in darkness are the only ones that have their affinity for the gas increased, or whether the elements which, under normal conditions of darkness, remain unoxidised are not also consumed in light. It is certain, however, that the amount of respiration in green cells increases *pari passu* with the intensity of the light, and at the higher intensities may reach such a degree as to kill the cell, and the light affects the contents directly and not through the medium of the green colour.

The degree of sensibility to light of the tissues of different plants varies greatly, and may be ascribed to the anatomical character of the contents and the dimensions of the illuminated cells. The great difference between green and non-green cells is specially noteworthy. The former are always more sensitive than colourless cells, and, indeed, than cells having any other colour besides green. It is, for example, more difficult to produce light paralysis and death in blue and colourless cells of *Tradescantia*, or in the filaments, sporangia, or oogonia of *Saprolegnia*, than in the larger cells of *Spirogyra* and *Nitella*, or in cells of the leaves of *Mnium* or *Vallisneria*.

Naturally the cause of difference is sought for in the green colouring matter, and such an explanation would be quite in accord with the relation of the colour to the action of light in assimilation as here demonstrated. As the seat of the action of light in assimilation is usually misplaced in the chlorophyll colouring matter, so likewise might the destruction of the cell-contents of the green cells in intense light be referred to the colouring matter as the starting point and, by its light absorption, agent of the decomposition, or, the cell-contents might be regarded as not sensitive to light, and the changes taking place in them as merely secondary effects of the destruction of the colouring matter. But such assumptions are improbable, and, indeed, are contradicted by the facts, that, red light, in spite of its stronger



absorption in chlorophyll, is inactive; that, the visible changes, whether paralysis or death, in intense light in the protoplasm of the cell are seen to be the direct effect of the light; and that, in non-green cells there is an undeniable light-effect. Direct proof that the colouring matter plays no necessary part is afforded in the possibility of destroying green cells by the insolation of a portion where there are no chlorophyll-corpuscles, *e. g.* in a pro-embryo or branch pro-embryo of *Chara*, where there are but few corpuscles lining the wall, or the part of a living *Nitella*-cell bared of chlorophyll corpuscles, as described on page 92. In such cases destruction proceeds as rapidly as it would if chlorophyll were present. Again, those species of *Spirogyra* are the most sensitive which have feebly coloured chlorophyll-bands, and the most widely separated bands. Cells with approximated and deeply coloured bands are not more strongly affected by light, as would be the case were the destruction of cell contents a consequence of that of the chlorophyll colouring matter; on the contrary, there is greater immunity from light effect. Differences in this respect are often very marked in one and the same species of *Spirogyra*. The effect of dissemination and aggregation of chlorophyll in retarding the action of light on the shaded part is easily observed in all green cells. The colouring matter is less sensitive to light than the other sensitive elements of the cell; and this is the case, as experiments in red light show, not only for lights of different intensities, but also of different spectral breadths.

The destruction, then, of protoplasm and the death of cells in light is a true light effect, independent of the destruction of chlorophyll colouring matter, taking place in green cells, as well as in those otherwise coloured, before complete destruction of the colouring matter, and it is not brought about through light-absorption in the chlorophyll-corpuscles. It is developed by absorption in the protoplasm itself of all the illuminating rays of the spectrum, the red rays up to those of wave lengths of  $\cdot 00061$  mm. being excluded from the action, and the chlorophyll colouring matter, instead of increasing the light-effect upon the cell contents of the part it shades, lessens the same. This, the evident, and, indeed, necessary effect of the colouring matter, has been hitherto entirely overlooked in estimating its physiological importance in respiration.

As death of the cell is not always accompanied by such striking changes as displacement of the nucleus, cessation of movement, &c., which are seen in *Spirogyra*, *Nitella*, and

*Tradescantia*, whilst the slightest colour-change is at once manifest, it is not always easy to detect the proper succession of changes in the cell which mark the difference in sensibility to light between the elements; but between the chlorophyll colouring matter with its vehicle, and the ground-substance and its contained matters this difference is very conspicuous. Hypochlorin disappears more quickly than chlorophyll colouring matter, and the ground-substance of the chlorophyll-corpuscles, after almost momentary exposure, loses all its vital peculiarities, whilst the colour remains still unchanged.

If, then, a green tissue is more sensitive to light than a not-green one, the cause does not lie in the colour but in the presence of easily-oxidisable assimilation-products which arise in the chlorophyll-corpuscles, and spread thence into the protoplasm. The action of light on the colouring matter is an incidental phænomenon, and the essential one is the far stronger destruction which the ground-substance of the chlorophyll-corpuscles and their included substances suffer. The chlorophyll-corpuscles are, therefore, extremely sensitive plates having a green screen. The actions stirred up within them by light are both reducing and oxidising, and for the latter their spongy construction and the possession of bodies such as oil and hypochlorin, easily converted into resins, renders them specially well fitted. To the green colouring matter no other share in these processes can be assigned than a physical one due to its colour; it diminishes the intensity of light, and thereby the amount of oxidation in the cell. This protection, however, fails in intense sunlight, as then the colouring matter is itself destroyed.

How far extends and wherein consists this protection under normal conditions of plant existence? Protection from danger to life of the cell from intense light has not to be considered. That is never or rarely required by the plant. It is only a protection of assimilation-products in the chlorophyll-corpuscles from too rapid destruction in daylight that has to be provided.

The amount of respiration in green tissues must necessarily from what has been said increase in daylight with increasing brightness; assimilation also rises in amount in light, but nearly reaches its maximum in medium day-brightness. Now, supposing the green tissues could perform their functions without chlorophyll colouring matter, the respiration would, in all intensities of daylight, and especially in the brighter light, greatly exceed in amount the assimilation (fig. 29 *c, d*). An accumulation of carbon would

then, even with this uninterrupted decomposition of carbonic acid, be quite impossible. The presence of chlorophyll colouring matter changes at once the condition to one favourable for such accumulation; for the absorption of oxygen increases in the more refrangible part of the spectrum—that part which is especially absorbed in the chlorophyll colouring matter—and proportionally too to the intensity of illumination. Even a single layer of chlorophyll-corpuscles in the cell absorbs in diffuse daylight, more or less strongly according to the depth of their coloration, all the blue up to the line F (fig. 28, spectrum  $\beta$ ), although in direct sunlight a considerable portion of the blue pass through. The amount of respiration in green tissues must, therefore, decrease in daylight, in consequence of their colour, and proportionally to its depth; and this, not only on account of a general reduction of illuminating power effected upon the whole spectrum, but specially through the selective absorption of the rays most refrangible and most active in respiration, which is characteristic of the chlorophyll colouring matter. In this way the respiration curve (fig. 29 *cf*) sinks in all higher intensities of light below that of assimilation (fig. 29 *ag*), for this latter process is but slightly influenced by reduction of light-intensity through the colouring matter, because it already has nearly reached its maximum in daylight of medium intensity, and also because the blue rays absorbed in the colouring matter are of less effect in destruction of carbonic acid. In daylight, chlorophyll colouring matter, by reducing the amount of respiration, allows assimilation to surpass it in amount, and thus enables an accumulation of carbon-compounds to take place; and in thus diminishing the respiration of green tissues in light lies the value of green colouring matter to plants.

Previous analytical researches have only slightly insisted upon this increased respiration in daylight. In green organs, as the concurrent assimilation always exceeds, except in the very lowest intensities, the respiration, it is necessarily, in light of the intensity of daylight, concealed. In spite of the great oxygen-absorption proceeding there is observed a constant giving off of oxygen only, and in order to make the increase of respiratory action evident by the accumulation of carbonic acid resulting from it, assimilation must either be suppressed or light (direct sunlight) of greater intensity must be employed. Sometimes, indeed, in direct sunlight the increment of respiration may be recognised, not by the accumulation of carbonic acid, but by the lessening of amount of the oxygen given off. Often observed this fact

has been misinterpreted. Famintzin,<sup>1</sup> for example, took this as proof of the diminution in amount of destruction of carbonic acid in sunlight, and of its being less than in bright diffuse daylight. But it is more rationally explained by an increased combustion in direct sunlight, so that the assimilation and respiration curves approach.

Tissues which are not green, and plants, such as phanerogamous saprophytes and Fungi, as they want the elements, especially the easily oxidised assimilation-products of the chlorophyll-corpuscles, which, in green cells, so readily absorb oxygen in light, are not so sensitive to light. A marked increase of carbonic acid accumulation does not take place in them, even in diffuse daylight of low intensity. Some researches in this direction by Drude<sup>2</sup> on *Monotropa*, and by Wolkoff and Mayer<sup>3</sup> upon germinating plants, have shown an increase in respiration in light. The latter found the differences very small, and considered them as tending to show that light had no important influence on respiration. But their results may be taken as supporting the theory here set forth. In experiments on respiration germinating seeds and green organs cannot be fairly compared as regards the substances used up. In the former it is the reserve materials—starch, fat, &c.—which, after metastatic change, are oxidised, whilst in the active green cells these substances, as has been shown, take no share in the respiration, but it is the primary assimilation-products or their immediate derivates which undergo combustion. If, therefore, with such unfavourable objects, an increase in the carbonic acid formation is observed, it is the more a distinct indication of the influence of light on respiration.

<sup>1</sup> "Melanges biologiques," 'Bull. d. l'Acad. Impér. d. St. Pétersbourg,' tom. x, 1880.

<sup>2</sup> 'Biologie der Monotropa Hipopitys,' Göttingen, 1873.

<sup>3</sup> 'Landwirthschaftliche Jahrbücher,' 1874.

(To be continued.)



## MEMOIRS.

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PRINGSHEIM'S RESEARCHES *on* CHLOROPHYLL.<sup>1</sup> Translated and condensed by Professor BAYLEY BALFOUR, of Glasgow University. (With Plates VIII and IX.)

(*Continued.*)

### V. *Assimilation and Colour.*

The known facts regarding assimilation in plants are not in opposition to the view here advocated, that the colour only indirectly, through respiration, takes part in this process, and that the colouring matter has no share in decomposing carbonic acid.

A. *Chemical Hypotheses of Chlorophyll Function.*<sup>2</sup>—Out of the general notion that chlorophyll colouring matter plays a direct part in assimilation, has developed the idea that its substance enters directly into the process of decomposition of carbonic acid, and that in this process it is constantly being destroyed and regenerated. This must be the basis of any chemical hypothesis of its function in assimilation. It not only assumes the destruction in light (and in daylight of medium brightness) of colouring matter, but also that this destruction is a consequence of appropriation of the carbon drawn from the carbonic acid decomposed. The carbon-compounds formed through assimilation in the plant body, would therefore be derived from the chlorophyll colouring matter as a mother-substance. This is, however, entirely hypothetical, and has no support from the side of organic chemistry, nor from direct experiment. The assumed genetic relations of the carbon-compounds to the chlorophyll

<sup>1</sup> 'Untersuchungen über Lichtwirkung und Chlorophyllfunction in der Pflanze.' Von N. Pringsheim, S. 152, mit 16 lithographirten Tafeln. Leipzig, 1881.

<sup>2</sup> See Sachsse: 'Die Chemie und Physiologie der Farbstoffe, Kohlenhydrate,' &c., Leipzig, 1877; and Wiesner, 'Die Entstehung des Chlorophylls,' Wien, 1877, for details regarding chemical hypotheses.

colouring matter are not explained, and, indeed, the constitution of the colouring matter—notwithstanding recent work on the so-called chlorophyll-crystals—is still as good as unknown. For the red and green crystals which can be extracted from artificial chlorophyll-solutions are by external characters, and by their spectra, proved to be in no way identical with the deeply-coloured green drops which exude from the chlorophyll-corpuscles, as described in this paper, or which, after the solvent has been removed, can be obtained from a solution of chlorophyll; and in them the normal colouring matter as it occurs in the tissues is obtained, still attached to its vehicle, from which, indeed, in the unchanged condition it has never yet been separated, and with which, after separation from the tissue, it is easily altered and converted into resin. This is the weak point in all chemical considerations of the genetic relations between chlorophyll colouring matter and the other contents of the cell, and at the present time the formation of carbo-hydrates out of the chlorophyll is entirely hypothetical. The destruction of chlorophyll colouring matter in daylight of medium intensity in which assimilation is possible, is quite unproved, improbable, and against all experience, and the whole chemical hypothesis based on such destruction of the chlorophyll colouring matter in light in direct connection with the decomposition of carbonic acid is quite untenable.

To the purely physical theory of chlorophyll-function here brought forward, the origin of the chlorophyll colouring matter and its genetic relations to the other cell-elements, are of incidental significance, but the facts adduced contradict decisively every theory based on the destruction of chlorophyll in the reducing process, showing—

1. Destruction of chlorophyll colouring matter in the living cell in light is an oxidation process independent of the presence of carbonic acid.

2. Chlorophyll colouring matter is not destroyed in light when in an atmosphere of carbonic acid and hydrogen in which assimilation is possible.

3. The destruction of chlorophyll colouring matter is a pathological process, and the colouring matter once destroyed is never regenerated.

B. *Is the Colouring Matter a necessary condition for Assimilation?*—Another theory, which regards chlorophyll colouring matter as a necessary condition for the decomposition of carbonic acid and water, assumes that it serves as a medium of transfer for the light. It absorbs rays of light which

are then passed on to and act upon the protoplasm of the cell, whilst the colouring matter itself remains unchanged. Such an hypothesis would be admissible only if it could be otherwise proved that the colouring matter takes part in the destruction of carbonic acid. But this by no means follows from our experience of gas-interchange in plants, and our knowledge of assimilation gives no support to such a theory, for the rays so strongly absorbed by the chlorophyll colouring matter are of no effect in assimilation.

That green tissues alone exhale oxygen in light may, at first sight, appear weighty evidence in favour of the colouring matter taking a direct part in the reduction-process, and it might find a very simple explanation in the absorbed rays being the source of energy. But when the change in amount of respiration in light is borne in mind, and also that the gas-interchange in green tissues is always the expression of the difference between assimilation and respiration, such an explanation is not satisfactory. The exhalation of oxygen by green tissues alone merely proves that in them respiration is less than assimilation, not that the green colour is an agent in the process.

Now, as under certain conditions of low light-intensity assimilation can proceed without any oxygen being exhaled, it is necessary to consider all conditions under which gas-interchange is taking place before measuring the amount of assimilation by the oxygen exhaled, or considering that the latter is always an index of the extent of the former. The old notion that assimilation only commences when there is a certain degree of brightness of illumination, because under some conditions of low light-intensity no oxygen is exhaled, is incorrect, as has been shown, and assimilation actually goes on at all, even the lowest intensities, but in the lower ones it is concealed by respiration. So that the exhalation of oxygen is only recognisable when respiration is less than assimilation.

It has been hitherto supposed that a plant only begins to assimilate when it becomes green, it is first green and then assimilates. But this idea has resulted again from the misconception that oxygen must be exhaled if there is assimilation. All that the facts warrant is, that respiration is less than assimilation only when the tissue is green, and consequently the exhalation of oxygen only commences with the appearance of green colour in the plant.<sup>1</sup>

All direct observations fail to confirm the theory that the

<sup>1</sup> Boussingault ('Comptes Rendus,' tom. 68, p. 410) has shown this is true from the very earliest appearance of colouring matter.



source of energy for the reduction-process lies in the colouring matter. Although the difficulties of accurate photometric determination of the absorption-spectra do not permit of complete proof, yet tolerably conclusive evidence against such a view is afforded by the facts that, the rays absorbed in the colouring matter, as indicated in the absorption-bands of the spectrum, which must be of some value to the plant, play no preponderating part in producing the light-effect upon the plant; that the maximum of decomposition of carbonic acid does not correspond with the maximum of absorption in the chlorophyll-spectrum; that leaves which are not active, show the same chlorophyll-spectrum as those which are active; and that artificial chlorophyll-solutions decompose no carbonic acid. The source of energy is to be sought for only in the light-absorption in the other cell-contents themselves, in which intense light brings about such marked decompositions.<sup>1</sup>

The sharing of the colouring matter in assimilation has been ere now questioned, though the grounds upon which this has been done have been in part incorrect. Thus, Meyer<sup>2</sup> and after him Mulder,<sup>3</sup> considered that the colouring matter instead of promoting assimilation is formed in the process. "Green tissues give out oxygen not because they are green but because they become green." This view has been repeatedly controverted,<sup>4</sup> for the transformation of the starch content of chlorophyll-corpuscles into wax, by which Mulder accounted for the liberation of oxygen, does not take place; and moreover, in the formation of chlorophyll in the plant oxygen is not set free. In later times,<sup>5</sup> Gerland has shown, in a discussion as to the relative energy of colours in assimilation, that the conformity of absorption-spectra of leaves with those in chlorophyll-solutions, and the decolorisation of the latter in oxygen, are not easily reconciled with the theory that colouring matter directly shares in assimilation.

It would appear, then, that the increase of respiration in light is retarded by the colouring matter, and that in this way the reduction of carbonic acid and water is favoured,

<sup>1</sup> That differences, dependent on their colour but difficult to define exist in their light-absorptions between active and non-active green tissues, has been proved by N. J. C. Müller, 'Bot. Untersuchungen,' Heidelberg, vol. i, and 'Handbuch der Allgemeinen Botanik,' 1880, vol. i, p. 511.

<sup>2</sup> 'Pflanzenphysiologie,' Bd. ii, p. 162.

<sup>3</sup> 'Allgemeine physiologische Chemie.' Uebersetzt von Kolbe, Braunschweig, 1844, p. 273.

<sup>4</sup> Mohl, 'Vermischte Schriften,' p. 360; Sachs, 'Experimental physiologie,' p. 320; De Bary, 'Bot. Zeit.,' 1871, p. 612.

<sup>5</sup> 'Ann. d. Phys. u. Chemie von Poggendorf,' vol. 143 (1871).



and an accumulation of carbon takes place in the plant, but the colouring matter takes no direct and immediate part in the process.

c. *How does this Theory of the Function of Chlorophyll affect the Result of Researches on Assimilation?*—As the oxidation and reduction-processes in green tissues do not rise and fall similarly in changing light-intensities and colours, it is necessary in all questions concerning assimilation to take into consideration the amount of respiration and the extent to which it is influenced by light. And

1. *As regards an optimum intensity of light for the decomposition of carbonic acid.*—This cannot be determined simply by the amount of oxygen given off in light, for this in all intensities is only the excess of oxygen exhaled over what is inhaled. Both processes, that of exhalation and that of inhalation of oxygen, are differently affected by light-intensity and colour, and if they are not distinguished from one another in a research the determination of the amount of oxygen exhaled fixes only approximately the relation between respiration and assimilation. It is possible in this way to determine only the intensities in which one or other process predominates, but not the amount of increase or decrease of decomposition of carbonic acid. The amount of oxygen exhaled, as has already been pointed out, is not an exact measure of the decomposition of carbonic acid, for an increase in brightness of illumination may bring an increase in decomposition of carbonic acid with an apparent decrease in the amount of oxygen given off (fig. 29, shows the approximation of the two curves in the highest intensities of daylight); and in this lies the explanation of the smaller amount of gas-evolution in direct sunlight than in bright diffuse daylight.

If now the exact carbon gain be sought instead of the amount of decomposition of carbonic acid in light, the oxygen exhalation again only gives an approximate result so long as the quantitative relation of the carbonic acid expired to the oxygen inhaled in varying intensities is unknown. The question is still further complicated by the unequal possession by the plants of chlorophyll, which must exercise an equally varying influence upon respiration and assimilation.

2. *The relative energy of the different rays of the spectrum in assimilation.*—This cannot be determined by the amount of gas given off in the different colours, because the absorption in the chlorophyll colouring matter, according to the screen-theory here set forth, must modify the result. All

accurate experimenters, from Daubeny<sup>1</sup> and Draper<sup>2</sup> to Sachs<sup>3</sup> and Pfeffer,<sup>4</sup> agree in showing that the greatest activity for evolution of oxygen by green tissues resides in the rays of middle refraction in the spectrum. Objections urged against this statement, and these come mainly from physiologists, who adopt a purely physical theory of assimilation, are essentially theoretical, based upon the idea that the colouring matter is the seat of light activity. Thus, Lommel considered that light action must be dependent on the degree of completeness of absorption of the rays and on their energy as measured by their heat-effect or mechanical intensity, and concluded that the chief activity must lie in the absorption-bands of the chlorophyll colouring matter, that is, in the red, because the blue, on account of their small mechanical intensity, could have no effect. Experiment does not, however, confirm this idea; and it were nearer the truth had the seat of activity been looked for in the cell-contents outside the chlorophyll colouring matter. Those physiologists who hold that yellow and green rays are more effective in decomposing carbonic acid than blue and red, rightly enough express the observed facts; but there remains for explanation the function of the rays so markedly absorbed in the colouring matter.

The experiments here recorded are not conclusive regarding the effects of colour in the reduction-process. Green and yellow are naturally more active than blue, because the latter is absorbed to such an extent by the chlorophyll colouring matter that it is unable to produce an effect, just as in photography the silver salts behind an interposed green glass-screen are much less sensitive to blue than to yellow and green light.

At the present time, then, notwithstanding many accurate researches already made, the dependence of the decomposition of carbonic acid upon the wave lengths of the light rays needs elucidation. There is no doubt that, for green plants, yellow and green rays are far more active for the evolution of oxygen than blue, yet this is no clue to the dependence of assimilation on colour. This process might equally well be stronger in the blue, for researches say nothing certain on this point. Yet *a priori* it appears more probable that blue rays have no effect, as the absorption in the chlorophyll colouring matter would be a more significant adaptation for the accumulation

<sup>1</sup> 'Philosophical Transactions,' 1836.

<sup>2</sup> 'Ann. d. Chem. et Phys.,' 1844.

<sup>3</sup> 'Bot. Zeit.,' 1864, p. 253.

<sup>4</sup> 'Arb. d. Bot. Instit. in Würzburg,' Part i, 1871.

of carbon in the plant if the absorption of the blue rays only enfeebled respiration without directly affecting assimilation.

Another outcome of the absorption in the chlorophyll colouring matter is, that the maximum activity in the spectrum for assimilation cannot be the same for all plants and for all brightnesses, but being dependent on the depth of colour of the plant and on the total intensity of illumination its position must change with both conditions. Herein may be found an explanation of the varying determinations which have been given of the course of the assimilation-curve in the spectrum.

3. *The constancy in volume* maintained in an atmosphere in which green plants are growing has been looked to as affording a clue to the chemical origin of the carbon-compounds produced in assimilation. The primary assimilation-products, it has therefrom been assumed, are directly derived from the carbonic acid and water in the reducing-process, the carbon of the former combining with the elements of water to form a carbo-hydrate whilst the oxygen is given off. And as anatomical evidence in support of this, the existence of starch in the chlorophyll-corpuscles, as well as the fancied physiological importance and distribution of glucose, have been quoted. The, at present, commonly received theory of assimilation then, which considers starch and sugar as the primary products of the process as opposed to the older one of Liebig that they are organic acids, is founded upon such considerations of gas-interchange supported by anatomical facts.

But although assimilation-theories are based upon this assumption (it may be noted in passing, how completely the nitrogenous constituents of the chlorophyll-corpuscles are shut out from any influence in the process, and there is, indeed, at present no reason for supposing that they have any), it is not a necessary consequence. Any such conclusions drawn from the constancy of the gas volume in gas-interchange would hold if green organs only assimilated and did not respire in light. But with respiration taking place the conditions must be different, because oxygen is thereby inhaled, and although this oxygen enters into combinations, one of the products of which is carbonic acid, yet the volume of the gases in this interchange are not equal. More oxygen is inhaled than carbonic acid exhaled. Germinating seeds rich in starch cannot, as has been pointed out, be simply or fairly compared with green organs of adult plants in respect of their respiration, but in germinating oily seeds more oxygen is evidently inhaled than carbonic acid exhaled;



and in green organs this inhalation of oxygen increases considerably in light. Respiration, then, in green organs exposed to light in a limited atmosphere necessitates a diminution in the gas-volume thereof.

If, now, the gas-volume around an assimilating and respiring plant remains constant, the immediate product of the reducing process must be a substance poorer in oxygen than a carbo-hydrate, and poorer by that amount of oxygen used up in the respiratory process. This conclusion is inevitable if the carbon-compounds are directly formed from the carbonic acid and water.

But, it might be supposed that the constancy of gas-volume observed under certain conditions only occurs if there is a definite amount of respiration, only if the primary reduction-product combining with oxygen is transformed almost entirely to a carbo-hydrate, which then persists as a stable reserve-substance in the chlorophyll-corpuscles resisting further oxidation in light. The amount of respiration in a green tissue would in that case influence not only the observed gas-interchange, but also determine the character of the formed compounds deposited within the chlorophyll-corpuscles. The function of these corpuscles is a double one, they assimilate and they respire ; and one is naturally led, on this account, to the hypothesis dealt with in the next chapter, that it is the accumulation of colouring matter which brings about the formation of different construction-products in the chlorophyll-corpuscles, that is to say, through respiration in the chlorophyll-corpuscle a primarily rich-in-carbon but poor-in-oxygen direct product of the reducing process passes into a more highly oxidised compound, the extent of oxidation being influenced by the amount of respiration in the corpuscle consequent upon the varying brightness of light reaching it, which in turn depends on the depth of colour in the tissues.

Chlorophyll colouring matter is thus by its absorption of so-called chemical rays, the constant regulator of respiration and assimilation, whilst its absorption in the red may perhaps increase the heat effect of these rays on the plant.

#### VI. *The Formation of Hypochlorin in Young Seedling Plants, and its Relation to Assimilation.*

From the point of view of the double function of chlorophyll-corpuscles enunciated in the last chapter, the formative substances found in them must be the result of the combined action of assimilation and respiration. Of the enclosed



bodies which are to be used up in metastasis, the chief are, starch, fat, perhaps sugar, tannin, and hypochlorin. All of them, it is here maintained, cannot be immediate products of the reducing process, probably hypochlorin is the primary assimilation-product. As every assimilation-theory must consider the origin and construction of all bodies enclosed in the chlorophyll-corpuscles, a word with regard to the connection of all these substances with chlorophyll-function may be said.

Controversy about the physiological value of these bodies has hitherto been confined to the question of the primary assimilation-product. Starch, having been for long the only highly-carbonised content of chlorophyll-corpuscles known, was considered as such, and its wide distribution, and, as Sachs<sup>1</sup> has clearly shown, its dependence upon light and carbonic acid supported this view. But starch, as is now known, is not the only, nor yet universal, but merely one of the most abundant products of assimilation. The like may be said of fat and sugar, each of which has been regarded as the first outcome of the reducing process, although the latter has never been proved to exist in chlorophyll-corpuscles, and also of tannin as it occurs in *Mesocarpus*, and indeed of all the ternary, rich-in-carbon, compounds hitherto known in the plant-body. The origin of each and all of these, not one of which is universally present in chlorophyll-corpuscles, is doubtless to be ultimately traced to the reduction in light of carbonic acid; but the only substance which is a constant and essential product of assimilation in the corpuscles is hypochlorin. The idea that the primary product of assimilation may vary in different plants, and that these substances may thus all be direct products under different conditions, is improbable and of no explanatory value. No fact positively forbids such a notion, but the similarity in structure and composition of the chlorophyll-corpuscles, and the great agreement in gas-interchange amongst green tissues, indicates an identity, in all, of the assimilation-process. Whatever theory be adopted there remains to be explained how it is that in one plant starch or fat or it may be both these substances, in another tannin or perhaps sugar, and in all hypochlorin, are formed and deposited inside the chlorophyll-corpuscles.

The theory here advanced is based upon the absorption of oxygen by the chlorophyll-corpuscles and upon the chemical

<sup>1</sup> 'Bot. Zeit.,' 1862, p. 365; 'Flora,' 1862, p. 165, 1863, "p. 193; Jahrb. f. Wiss. Bot.,' iii (1863) p. 199; 'Bot. Zeit.,' 1864, p. 289; Experimental Physiologie,' p. 133.

nature of their included bodies. These all agree chemically in this, that they are non-nitrogenous, and they are visible products of assimilation, derivates, differing from one another in oxygen-content, of the carbonic acid and water decomposed in the process, the extent of their oxygenation being determined, on the hypothesis of a single primary assimilation-product, by the amount of respiration in the corpuscles as governed by the intensity and colour of light. From the side of chemistry this view is not contradicted, but its admissibility depends on anatomical and physiological considerations.

The primary reduction-product, of which, by oxidation, the ternary compounds in the chlorophyll-corpuscles are in the widest sense derivates, is to be sought for in the drops exuded from the corpuscles after treatment with dilute acid or moist warmth, as these contain all the constituents of the corpuscle sensitive to light, and with strong affinity for oxygen. All the nitrogenous compounds and stable ternary assimilation-products—starch, fat, tannin, &c.—remain intact within the corpuscles. In the exuded mass, hypochlorin, which is the only very sensitive substance besides the colouring matter itself, is present as is known. Its universal occurrence has already been referred to. Wherever chlorophyll occurs it is to be found; so constant, indeed, is this, that in epidermal cells and hairs of phanerogams, or the cells of phanerogamic parasites, in which chlorophyll-corpuscles are exceedingly sparsely distributed, their assimilatory activity may be readily proved by the demonstration of hypochlorin through treatment with acid or other suitable reagent. It is found along with and without the other constituents above mentioned. They may be derived from it; it cannot arise from them. Its sporadic occurrence in the chlorophyll-corpuscles of one and the same cell indicates its employment in assimilation. The accumulation of deposits of formative material, especially starch, in the chlorophyll-corpuscles, increases with age; the hypochlorin, on the other hand, decreases in the green tissue as they grow older. Indeed, the richer they are in these deposits the poorer are they in hypochlorin. All this points strongly to the genesis of these bodies out of hypochlorin, and equally so does the constant relation of hypochlorin in *Spirogyra* and other *Conservee* to the amylum-bodies where a causal connection can hardly be denied.

Hypochlorin, then, is not only universally present as a product of the chlorophyll-corpuscles, but has also a very definite time and place-relation to the formation of the

substances deposited therein. It is the primary product of the reduction-process, and is the basis of all the ternary compounds in the corpuscles.

Further experimental proof of this is found in the formation of hypochlorin in young seedling plants under the influence of light. Seedlings of angiosperms grown entirely in the dark are not green and have no hypochlorin. The duration of culture in darkness matters not. Hypochlorin cannot be formed in this instance out of the reserve materials in the plant, and therefore, even if seedlings grow in darkness until all the reserve material is used up, no hypochlorin is formed. Light is necessary for its production, and that, too, of a greater intensity than is necessary for development of green colour in the seedling. Both the hypochlorin and the chlorophyll colouring matter only arise if the seedling when still developing is placed in light, and the hypochlorin appears later than the chlorophyll colouring matter, and after a longer exposure to the light.

Seedlings of such plants as peas, hemp, cucumber, flax, &c., which, after being grown for eight days in the dark are still capable of becoming green and of developing, if exposed in a temperature of  $20^{\circ}$  to  $23^{\circ}$  C. (in July and August), to a bright diffuse daylight, become plainly green in two to three hours, and in six to ten hours are very deep green, but only after some nineteen or twenty hours of exposure are traces of hypochlorin found.<sup>1</sup> Hypochlorin in seedlings of angiosperms is thus only formed under the influence of light, and as a consequence of assimilation,

<sup>1</sup> Table showing relative time of appearance of Hypochlorin and Chlorophyll in Seedlings of Angiosperms grown in the Dark, and the dependence of Hypochlorin upon Light. The experiments were made upon seedlings of peas, hemp, cucumbers, flax, and other plants, all from seeds sown at same time.

| Age, in days, of Seedlings. | Exposure to Light, in hours. | Character of Light.     | Colour of Seedlings.     | Hypochlorin.          |
|-----------------------------|------------------------------|-------------------------|--------------------------|-----------------------|
| 8—13                        | 0                            | ...                     | Deep yellow <sup>2</sup> | None.                 |
| 8                           | 6                            | ...                     | Distinctly or deep green | None.                 |
| 8                           | 13—16                        | Bright diffuse daylight | Deep green               | None.                 |
| 8                           | 19—20                        | ...                     | Deep green               | Sparing first traces. |
| 8                           | 30—31                        | Full daylight           | Deep green               | In all tissues.       |

<sup>2</sup> In *Cucurbita* the cotyledons were, as is often the case in this plant, bright green.



and only after prolonged exposure, and when the plant has become very deep green. This very significant fact may be explained by the relation of the colouring matter to respiration. The formation of hypochlorin in the plant begins with the commencement of illumination. How, then, is evidence of this effect of light not apparent in the first twenty hours? Because apparently the hypochlorin is consumed in light until there is a sufficient accumulation of colouring matter for its protection, and it is also possible that the hypochlorin first formed may be converted into colouring matter and may thus be the mother substance of the chlorophyll colouring matter, an hypothesis which, as will presently be pointed out, may explain the development of green colour in the dark within the tissues of gymnospermous embryos.

The relation of hypochlorin-formation to assimilation and respiration may be further elucidated by placing the seedlings grown in the dark not in bright full daylight, but in conditions of half darkness (a darkened room). Here they become quite green, but do not live long, and disintegrate almost as rapidly as those growing in complete darkness. Such facts have led to the supposition which has been already refuted in this paper, that a plant becomes green in light of a lower intensity than is requisite for assimilation. The explanation really is that, in the relations of assimilation to respiration, this low intensity is unfavourable for assimilation, and the products of this process are, without any permanent gain to the plant, again used up. If seedlings grown in darkened rooms are brought into bright light, which is favourable to development of green colour but not to evolution of free oxygen, then no hypochlorin is to be found in them. Such plants grown in half-dark conditions whether they have been in half darkness throughout or were, at first in complete darkness) show no trace of hypochlorin, even if the plant is as well formed and as deeply green as a seedling which has for some days grown in full daylight and contains abundance of hypochlorin. It depends on the regulation of the brightness whether or no after a time, say eight to fourteen days, hypochlorin is found in plants grown under half-dark conditions; for as soon as assimilation is greater than respiration then hypochlorin accumulates and increases in amount with the increasing brightness. Beautiful green seedlings destitute of hypochlorin may be grown under a glass shade covered with grey paper in the half-dark illumination on the side farthest from the light of a deep room. Its absence from these is a proof that it is used up



in respiration so long as that process in light is in excess of assimilation. Hypochlorin-formation, therefore, is fully proved to depend upon light. Its dependence upon the presence of carbonic acid is difficult of experimental proof, because, though seedlings may be cultivated in an atmosphere deprived of carbonic acid, it is impossible by absorptive agents to keep it free of the gas; as in presence of oxygen, which is here necessary for the development of the green colour in the tissues, the time that must elapse before the hypochlorin-formation can be detected is sufficient for carbonic acid to accumulate within the tissues and to so great an extent as to give rise to hypochlorin.

Of very striking import is the fact that hypochlorin, like chlorophyll colouring matter, is formed in gymnosperms without the action of light. Colourless embryos of *Pinus picea*, *montana*, *maritima*, *Larix*, have no trace of hypochlorin. Sachs showed that from the seeds of such plants germinating in darkness, the embryos, though kept quite dark, become green. This is quite unexplained. In the first stages of germination, when the seedling is already green, no hypochlorin is formed; but in later stages, though still in darkness, it appears in the tissues. Thus, hypochlorin appears in gymnosperms grown in darkness, and, as is the case in angiosperms after the chlorophyll colouring matter, in most cases appearing in four to five weeks' old seedlings, which have become green long before.<sup>1</sup> There is no doubt, however, that light favours hypochlorin formation.

This condition in gymnosperms does not disallow the hypothesis that hypochlorin is formed in assimilation. Although formed in the embryo without access of light, yet in adult gymnosperms as in angiosperms it is the result of light-action. It resembles chlorophyll in this respect. Because chlorophyll occurs in embryos of gymnosperms grown in the dark, one does not suppose that light has no influence in its production in other plants, and the like must be held regarding hypochlorin. Possibly in seedling-gymnosperms the hypochlorin may arise by metastasis without direct assimilation. A substance—perhaps a volatile oil—may descend from the mother-plant into the seeds, and out of it the hypochlorin in the seedling may be formed. Whatever be the first assimilation-product it is possible that it may be regenerated by metastasis from its own products, and for all proximate constituents of the plant the same process of regene-

<sup>1</sup> The following Table illustrates the formation of Hypochlorin in Seedlings of various ages of *Pinus picea*, grown in Darkness, some being exposed

ration is possible. But in respect of its origin, hypochlorin appears more strongly bound up with assimilation than these other proximate constituents of the plant body; for, of all the products in the chlorophyll-apparatus, hypochlorin is the only one besides the chlorophyll colouring matter itself, which, in angiosperms, cannot develop without light.

Complete anatomical proof that hypochlorin is the primary assimilation-product is not yet possible, our knowledge of it is too recent, and it is only by artificial imitation of the assimilation-process that all doubt can be set at rest. Yet the close relation of hypochlorin with the function of the chlorophyll-corpuscle—with its assimilation and respiration—has been fully and with certainty established through the foregoing account of its origin, its constant occurrence in chlorophyll-corpuscles, and its behaviour in light and oxygen. With no body in the cell does the hypochlorin exhibit such close relationships of function as with chlorophyll colouring matter. So much so, indeed, as to almost lead to the belief that it is an artificial product of the chlorophyll colouring

*for a longer or shorter time to Light. All the Seedlings at the time of examination were deep green.*

| Age, in days, of Seedlings. | Days exposed to Light. | Number of Seedlings Examined. | Presence or Absence of Hypochlorin.                    |
|-----------------------------|------------------------|-------------------------------|--------------------------------------------------------|
| 12                          | 0                      | 3                             | None.                                                  |
| 12                          | 0                      | 3                             | In two seedlings, none; in the other, perhaps a trace. |
| 15                          | 0                      | 3                             | In two, none; in one, a little.                        |
| 17                          | 0                      | 1                             | None.                                                  |
| 18                          | 1                      | 1                             | A trace.                                               |
| 19                          | 0                      | 1                             | None, or perhaps a trace.                              |
| 19½                         | 2½                     | 1                             | Some, but not much.                                    |
| 21                          | 1                      | 1                             | A trace.                                               |
| 22                          | 0                      | 1                             | None, perhaps a trace.                                 |
| 22                          | 0                      | 3                             | Some, but little.                                      |
| 23                          | 3                      | 1                             | Considerable amount.                                   |
| 24                          | 5                      | 1                             | Considerable amount.                                   |
| 26                          | 0                      | 6                             | In four, none; in two, some.                           |
| 26                          | 7                      | 4                             | A large amount.                                        |
| 28                          | 0                      | 1                             | A trace.                                               |
| 28                          | 0                      | 3                             | In two, none; in one, a little.                        |
| 28                          | 8                      | 1                             | A large amount.                                        |
| 33                          | 11                     | 1                             | A large amount.                                        |
| 39                          | 0                      | 6                             | In three, a trace; in three, much.                     |
| 39                          | 7                      | 6                             | A large amount.                                        |

Still more marked than in this species is hypochlorin found in four weeks' old seedlings of *P. maritima* grown in darkness.

matter developed by the reagents used. This, however, is not the case. The constant association of hypochlorin and colouring matter, and the difficulty of separating them, would suggest, as has been already hinted, a genetic connection between them. Nothing positive about this is as yet known. Hypochlorin exists always and only in chlorophyll-corpuscles because it is a product of these and of the function of their colouring matter, as its formation in light depends on the presence of the colouring matter. Their analogous circumstances of origin are very striking, especially their formation in the dark in gymnosperms, seeing that both are otherwise dependent on light. Possibly a common origin may be assumed for both in gymnosperms. If a connection between them were established, and the chlorophyll colouring matter was developed from the hypochlorin, it would then have to be considered an assimilation-product. This is by no means inconceivable. The grounds upon which one denies the possibility of chlorophyll colouring matter arising in consequence of assimilation are no argument against it. That chlorophyll colouring matter is a preliminary condition of assimilation has been already refuted and its true function proved. The development of the green colour of gymnosperms in darkness, and the formation of chlorophyll in an atmosphere free of carbonic acid, are as little opposed to it as to the view that hypochlorin is a product of assimilation. That development of green colour precedes assimilation has been shown to be founded on a misinterpretation of appearances. Taking all these facts together one can hardly shake off the impression that the development of green colouring matter is one of the immediate effects of assimilation. The exact time of development of green colour coincides very nearly with that of the first evolution of oxygen from the tissues, yet after the facts brought forward assimilation must always precede the evident evolution of oxygen. Where light falls on a plant hypochlorin and chlorophyll arise together.

According to the theory of the function of chlorophyll now advanced, the green colour of plants is a natural adaptation to the needs of assimilation, and the origin of a protecting screen of colouring matter from hypochlorin, the accumulation of which in light is one of the advantageous results of assimilation, would appear to satisfy these needs in a simple and appropriate manner.

VII. *Results in detail of some Experiments.*

For the production of the coloured lights employed in these experiments the following solutions were used :

|          |   |   |   |                                 |
|----------|---|---|---|---------------------------------|
| For red  | . | . | . | iodine in bisulphide of carbon. |
| „ yellow | . | . | . | potassium bichromate.           |
| „ green  | . | . | . | copper chloride.                |
| „ blue   | . | . | . | ammonia copper sulphate.        |

Their spectra are described on pages 87 and 88, and are represented in fig. 28.

Successive experiments in each group followed one another immediately, sufficient time—usually only five to ten minutes—being allowed for adjustment of the apparatus and object. In order to facilitate recognition of the cells, especially in experiments where no effect is visible, the bending of the object in a V-shaped manner into a long and short leg is useful. Where as in experiments 21 and 22, slight effect is observed from light in an atmosphere of hydrogen, it is due either to the long exposure of the cells permitting the development of much heat, and is therefore a thermal effect, or it may be that the hydrogen has not completely expelled all the oxygen from the gas chamber—not having been introduced sufficiently long before commencement of the experiment. It should begin to pass through the chamber about half an hour before insolation. It is further to be noted that hair-cells of *Tradescantia* and coloured cells which are not green are with difficulty destroyed in light; after eighteen to twenty minutes in feebly-intense coloured light they remain intact. It must be also remembered that aerial parts do not respire freely under water, and can only take up to a small degree oxygen dissolved in water. They are, in fact, almost in the conditions of an oxygen free chamber. It is, therefore, more difficult to kill them under such conditions by light, *i.e.* they require longer exposure than do green cells of water-plants. Such long duration of exposure may, however, induce heat-effect, and it is therefore well to lay carefully the object so that only one side of the cells is submerged, the other side being exposed to the atmosphere.

The details regarding the experiments arranged in the following tables sufficiently explain themselves:



## I. First Series.—Comparative Experiments with Differently-coloured Concentrated Light in Atmospheric Air.

| Group of Parallel Experiments. | Plant.                   | Number of Experiment. | Colour of Light. | Spectrum, see Fig. 28. | Intensity of Light. | Duration of Insolation. | Results.                                                                                                                                                                                                                                                                                                            |
|--------------------------------|--------------------------|-----------------------|------------------|------------------------|---------------------|-------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| A.                             | <i>Spirogyra crassa</i>  | 1                     | Blue             | $\epsilon$             | Feeble              | 5 min.                  | Strong effect. Chlorophyll-bands of exposed cells completely decolorised. Motion of granules stopped. Central plasma swollen and vesicular. Nucleus, at first central, soon displaced. Plasma-threads ruptured. Cell dead.                                                                                          |
|                                |                          | 2                     | Red              | $\delta$               | Strong              | 7 min.                  | No trace of light-action immediately or subsequently. Colour, form, arrangement, and character of cell-contents completely retained.                                                                                                                                                                                |
|                                |                          | 3                     | Green            | $\theta$               | Strong              | 5 min.                  | Strong effect. Chlorophyll-bands almost entirely decolorised and contracted. Central plasma swollen and vesicular. Shortly before end of experiment nucleus displaced. Plasma-threads ruptured. Cell dead.                                                                                                          |
| B.                             | <i>Spirogyra jugalis</i> | 4                     | Red              | $\delta$               | Strong              | 10 min.                 | No trace of action. Also some hours later the cells and contents quite unchanged and normal.                                                                                                                                                                                                                        |
|                                |                          | 5                     | Red              | $\delta$               | Strong              | 14 min.                 | No trace of action. Later unchanged.                                                                                                                                                                                                                                                                                |
|                                |                          | 6                     | Blue             | $\kappa$               | Strong              | 6 min.                  | Chlorophyll-bands decolorised. Central plasma and nucleus swollen, vesicular, and displaced. Plasma-threads ruptured, partly disappeared. Cell dead.                                                                                                                                                                |
| C.                             | <i>Spirogyra jugalis</i> | 7                     | Red              | $\delta$               | No effect.          | 16 min.                 | No effect. And later remaining unchanged.                                                                                                                                                                                                                                                                           |
|                                |                          | 8                     | Green            |                        | Feeble              | 7 min.                  | Powerful effect. Chlorophyll-bands decolorised. Central plasma and nucleus swollen, vesicular, and displaced. Plasma-threads ruptured. Cell dead.                                                                                                                                                                   |
|                                |                          | 9                     | Red              | $\delta$               | Strong              | 20 min.                 | No evident effect. Chlorophyll-bands retain colour, form, and disposition. Central plasma, nucleus, and plasma-threads unchanged in form, disposition, and character; but motion of granules arrested. Two hours later cell quite healthy and normal, and motion of granules had recommenced. Cell remained normal. |

## I. First Series—continued.

| Group of Parallel Experiments. | Plant.                                                                       | Number of Experiment. | Colour of Light. | Spectrum, see Fig. 25. | Intensity of Light.             | Duration of Insolation. | Results.                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|--------------------------------|------------------------------------------------------------------------------|-----------------------|------------------|------------------------|---------------------------------|-------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| D.                             | <i>Nitella flexilis</i>                                                      | 10                    | Green            | $\theta$               |                                 | 3 min.                  | Strong effect. Decolorisation at insolated area marked but not complete. Rotation normal. Arrangement of chlorophyll-corpuscles in rows undisturbed. After many days same normal condition obtains (fig. 23), but decolorised chlorophyll-corpuscles of insolated area separated from wall and rotate in the current with other contents. Cell remains in this condition for months. No formation of new chlorophyll-corpuscles at insolated area. |
| E.                             | <i>Nitella flexilis</i>                                                      | 11                    | Red              | $\delta$               |                                 | 16 min.                 | No trace of effect of any kind.                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                |                                                                              | 12                    | Green            | $\theta$               |                                 | 5 min.                  | In all more or less strong effect. Decolorisation of insolated area. Slowing of the rotation, and later death of the cell.                                                                                                                                                                                                                                                                                                                         |
|                                |                                                                              | 13                    | Yellow           | $\epsilon$             |                                 | 5 min.                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|                                |                                                                              | 14                    | Blue             | $\iota$                |                                 | 5 min.                  | No trace of effect either immediately or later.                                                                                                                                                                                                                                                                                                                                                                                                    |
| F.                             | <i>Nitella mucronata</i><br>Leaf segments used 2.5 mm. long, 0.12 mm. thick. | 15                    | Red              | $\delta$               | Feeble                          | 15 min.                 | Strong effect. Decolorisation strong, not quite complete. Rotation arrested. Organisation retained. Later: rotation not recommenced.                                                                                                                                                                                                                                                                                                               |
|                                |                                                                              | 16                    | Blue             | $\iota$                |                                 | 5½ min.                 | Disorganisation begun.                                                                                                                                                                                                                                                                                                                                                                                                                             |
|                                |                                                                              | 17                    | Green            | $\zeta$                | Feeble, but stronger than last. | 8 min.                  | Feeble effect. Decolorisation slight, not extended. Rotation persists. Order retained. Later: rotation and order retained. Cell remains alive.                                                                                                                                                                                                                                                                                                     |
|                                |                                                                              | 18                    | Green            | $\zeta$                |                                 | 6 min.                  | Small effect. Decolorisation slight. Rotation quite persists. Order retained. Chlorophyll-corpuscles of insolated area turned edgewise to the light. Cell remains alive.                                                                                                                                                                                                                                                                           |
|                                | Leaf segments used 5.0 mm. long, 0.15 mm. thick.                             | 19                    | Blue             | $\iota$                |                                 | 6 min.                  | Strong effect. Decolorisation extended, complete. Rotation extremely feeble. Aggregation of irregular protoplasm-masses at insolated area. Later: rotation completely arrested. Commencing disorganisation.                                                                                                                                                                                                                                        |

## II. Second Series.—Comparative Experiments with Concentrated Light in Different Gases.

| Group of Parallel Experiments. | Plant.                   | Number of Experiment. | Atmosphere.  | Colour of Light. | Spectrum. See Fig. 28. | Duration of Insolation.                    | Results.                                                                                                                                                                                                                                                                                                                                                                          |
|--------------------------------|--------------------------|-----------------------|--------------|------------------|------------------------|--------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| A.                             | <i>Spirogyra crassa</i>  | 20                    | Atmosph. air |                  |                        | 4 min.                                     | Extended effect, which, as often happens in white light, reaches adjacent non-insolated cells. Chlorophyll-bands in insolated cells completely, in non-insolated partly, decolorised. Central plasma with nucleus swollen, vesicular, and displaced. Plasma-threads ruptured, disappeared, their remains with plasma-knots here and there attached to central plasma (fig. 11 b). |
|                                |                          | 21                    | Hydrogen     | White            | $\alpha$               | 8 min.                                     | No effect at first. Later, after some hours: here and there very slight effects in insolated cells, <i>e.g.</i> plasma-knots on the threads (fig. 11 a). But colour of chlorophyll-bands remains.                                                                                                                                                                                 |
|                                |                          | 22                    | Hydrogen     |                  |                        | 9 min.                                     | No trace of effect immediately seen. Later, as in 21: slight changes in plasma-threads or projections on chlorophyll-bands.                                                                                                                                                                                                                                                       |
|                                |                          | 23                    | Atmosph. air |                  |                        | 3 min.                                     | Extended effect passing, as in 20, beyond limit of insolated area. Chlorophyll-bands completely decolorised. Central plasma with nucleus swollen, vesicular, and displaced. Plasma-threads ruptured, disappeared. Cell dead.                                                                                                                                                      |
|                                |                          | 24                    | Hydrogen     |                  |                        | 20 min. Sun for a minute slightly obscured | No trace of effect, immediately or subsequently, even next day.                                                                                                                                                                                                                                                                                                                   |
| B.                             | <i>Spirogyra jugalis</i> | 25                    | Atmosph. air | Green            | $\theta$               | 5 min.                                     | Complete effect. Chlorophyll-bands decolorised. Central plasma and nucleus swollen, vesicular, and displaced. Plasma-threads coagulated and half ruptured. Cell dead                                                                                                                                                                                                              |

## II. Second Series—continued.

| Group of Parallel Experiments. | Plant.                   | Number of Experiment. | Atmosphere.                                               | Colour of Light.                    | Spectrum. See Fig. 28.                                 | Duration of Insolation. | Results.                                                                                                                                                                                                                                                                                                              |
|--------------------------------|--------------------------|-----------------------|-----------------------------------------------------------|-------------------------------------|--------------------------------------------------------|-------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| C.                             | <i>Nitella flexilis</i>  | 26                    | Hydrogen Atmosph. air                                     | Green (thro' green glass over lens) |                                                        | 20 min.                 | No trace of effect. Powerful effect. Insolated part of cell completely decolorised. Rotation stopped. Plasma aggregated in the middle. Two currents arise in cell. The non-decolorised chlorophyll-corpuscles in upper part begin later to swell.                                                                     |
|                                |                          | 27                    |                                                           |                                     |                                                        | 7 min.                  |                                                                                                                                                                                                                                                                                                                       |
| D.                             | <i>Spirogyra crassa</i>  | 28                    | Pure hydrogen                                             | Green                               | $\theta$                                               | 7 min.                  | No effect.                                                                                                                                                                                                                                                                                                            |
|                                |                          | 29                    | Mixt. of CO <sub>2</sub> and H, 1 CO <sub>2</sub> to 20 H |                                     |                                                        | 7 min.                  | No effect. Only the nucleus slightly pushed from the centre.                                                                                                                                                                                                                                                          |
|                                |                          | 30                    | Mixt. of CO <sub>2</sub> and H, 1 CO <sub>2</sub> to 10 H |                                     |                                                        | 10 min.                 | No effect.                                                                                                                                                                                                                                                                                                            |
|                                |                          | 31                    | Atmosph. air                                              |                                     |                                                        | 7 min.                  | Powerful effect. Chlorophyll-bands decolorised, but retaining form and disposition. Central plasma with nucleus swollen, vesicular, ruptured, and displaced. Plasma-threads ruptured, partly destroyed, &c.                                                                                                           |
| E.                             | <i>Nitella macronata</i> | 32                    | Atmosph. air                                              | Blue                                | $\kappa$                                               | 10 min.                 | Enormous effect. Complete and extended decolorisation of insolated area. Permanent stoppage of rotation. Collapse of protoplasmic utricle. Destruction in the streaming plasma. Swelling of non-insolated non-decolorised chlorophyll-corpuscles throughout the whole cell.                                           |
|                                |                          | 33                    | Hydrogen Atmosph. air                                     | Blue                                | $\kappa$                                               | 13 min.                 | No trace of effect.                                                                                                                                                                                                                                                                                                   |
|                                |                          | 34                    |                                                           | Green                               | Wider than $\theta$ from wave lengths .00059 to .00044 | 7 min.                  | Powerful effect. Chlorophyll-corpuscles of insolated area decolorised. Rotation in whole cell arrested. Plasma aggregations at insolated area. Arrangement of the protoplasm utricle and the rows of chlorophyll-corpuscles retained. After some hours, disorganisation and collapse of plasma-utricle. Cell is dead. |



|    |                                     |    |                                |                            |   |         |                                                                                                                                                                                                                                                                                                                               |
|----|-------------------------------------|----|--------------------------------|----------------------------|---|---------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| F. | <i>Nitella</i> and <i>Spirogyra</i> | 35 | Hydrogen                       | Green                      | " | 12 min. | No trace of effect either immediate or subsequent.<br>No trace of effect either immediate or subsequent. No heat effect.                                                                                                                                                                                                      |
|    |                                     | 36 | Hydrogen                       | Green                      | " | 20 min. |                                                                                                                                                                                                                                                                                                                               |
|    | <i>Nitella</i> and <i>Spirogyra</i> | 37 |                                | Blue                       | κ | 6½ min. | In all, extended and complete effect, as in ordinary atmospheric air. Decolorisation at insolated area. Greater or less destruction in protoplasm. Displacement of nucleus in <i>Spirogyra</i> , and rupture of plasma-threads. Temporary or permanent arrest of rotation, and disorganisation in <i>Nitella</i> .            |
|    |                                     | 38 |                                | Blue, thro' a blue glass   |   | 7 min.  |                                                                                                                                                                                                                                                                                                                               |
|    | <i>Nitella</i> and <i>Edogonium</i> | 39 | Air deprived of carbonic acid. | Green, thro' a green glass |   | 7 min.  |                                                                                                                                                                                                                                                                                                                               |
| G. | <i>Nitella</i> and <i>Spirogyra</i> | 40 |                                | Green                      | θ | 8½ min. | Strong effect. <i>Insolated cells decolorised</i> , dead (fig. 20 c, b). Previously straight filament ruptures at insolated part. Insolated cells separate one from another. The tannin-vesicles ruptured and contents disappeared, pellicles appressed to contracted chlorophyll-plate, giving it frothy appearance.         |
|    | <i>Mesocarpus scalaris</i>          | 41 | Atmosph. air                   | Blue                       | κ | 3 min.  |                                                                                                                                                                                                                                                                                                                               |
|    |                                     | 42 | "                              | Green                      | θ | 3 min.  | Strong effect, as in 41. <i>Chlorophyll plate decolorised</i> . Later: cells separate, and tannin-vesicles, at first still visible, soon also disappear. Cell dead.                                                                                                                                                           |
|    |                                     | 43 | "                              | Red                        | δ | 5 min.  | Strong effect (perhaps purely thermal?). <i>Chlorophyll in insolated cells completely retained</i> (fig. 21, a, b, c, d). Otherwise chlorophyll-plate contracted strongly at insolated area. Tannin-vesicles vanished early, but pellicles with collapsed utricle forming froth-like coating to chlorophyll-plate. Cell dead. |

## II. Second Series—continued.

| Group of Parallel Experiments. | Plant.                                                                                                                                                             | Number of Experiment. | Atmosphere.  | Colour of Light. | Spectrum. See Fig. 25. | Duration of Insolation. | Results.                                                                                                                                                                                                                                                                                                             |
|--------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|--------------|------------------|------------------------|-------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| G.                             | <i>Mesocarpus scalaris</i> —continued.                                                                                                                             | 44                    | Hydrogen     | Green            | $\theta$               | 10 min.                 | No immediate effect. Later : no effect.<br>No immediate effect. Later : slight destruction as in red light. Chlorophyll remains undestroyed, but cell cohesion lost and tannin-vesicles disappeared.                                                                                                                 |
|                                |                                                                                                                                                                    | 45                    |              | Green            | $\theta$               | 12 min.                 |                                                                                                                                                                                                                                                                                                                      |
| H.                             | <i>Tradescantia Selowii</i> .<br>Nine-celled hair from stamen used. Cells 1, 2, 3, 4, insulated, 5 partly insulated, rest shaded. Hair in water under cover glass. | 46                    | Atmosph. air | Green            | $\eta$                 | 22 min.                 | Immediate paralysis at insulated area. Circulation of insulated cells arrested. In one or two adjacent cells, circulation feeble. In rest, circulation vigorous. Later : recovery. In all cells vigorous circulation.                                                                                                |
| I.                             | <i>Tradescantia virginica</i> .<br>Object in this group not covered by water-drop. Stamen-hair.                                                                    | 47                    | Atmosph. air | Blue             | $\kappa$               | 15 min.                 | Immediate effect feeble. Insolated cells retain colour. Circulation arrested. Order retained. Non-insolated cells normal. Later : effect strong. Colour of insulated cells destroyed—cells violet. Plasma-threads coagulated or ruptured. Cell dead. Non-insolated cells normal.                                     |
|                                | Stamen - hair and pollen-grain.                                                                                                                                    | 48                    | Atmosph. air | White            | $\alpha$               | 5 min.                  | Immediate effect strong. Insolated cells (fig. 27, $\gamma$ , $\delta$ , $\epsilon$ , $v$ , $w$ , $x$ , $y$ ) become violet, and later, lose entirely colour. Plasma-threads coagulated, circulation stopped, often ruptured. Later : cell content collapsed. Cell dead. Yellow pollen-grain ( $p$ ) retains colour. |

|                                                       |    |              |       |   |         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|-------------------------------------------------------|----|--------------|-------|---|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Stamen-hair.                                          | 49 | Hydrogen     | White | " | 14 min. | No effect. Only slight temporary arrestment of circulation in insulated cells.                                                                                                                                                                                                                                                                                                                                                                                                                |
| "                                                     | 50 | Hydrogen     | White | " | 15 min. | No effect. Ditto, ditto.                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Three cells entirely, and two cells partly insulated. | 51 | Atmosph. air | White | " | 10 min. | Immediate very strong effect. Insolated cells have completely lost colour. Order lost. Plasma-threads coagulated, ruptured. Cells dead. One partially insulated, cells are violet; content collapsed. Cell dead. Other partially insulated, cell is violet; order destroyed, circulation arrested. Cell dead. Non-insolated cells are violet, order destroyed, circulation arrested. Cell dead. Rest normal. Here effect has extended beyond insulated area, as it often does in white light. |
| Two stamen-hairs.                                     | 52 | Atmosph. air | White | " | 5 min.  | Hair 1. Immediate: insulated cells violet. Plasma-threads paralysed, and circulation stopped. In one adjacent cell circulation stopped. Later, after four hours: insulated cells decolorised, dead. All others normal.<br>Hair 2. Immediate: insulated cells violet. Plasma-threads ruptured. In two partly insulated cells circulation arrested. Rest normal. Later: insulated and partly insulated cells decolorised. Plasma partly destroyed. Cells dead. Rest normal.                     |

*The DEVELOPMENT of ARTICULATED LATICIFEROUS VESSELS.*  
By D. H. SCOTT, B.A., Ph.D. (With Plate X.)

1. *Literature.*

THE development of laticiferous vessels from cells was, as regards certain instances, known to the younger Moldenhawer<sup>1</sup> as early as the year 1812. At that time these organs were not yet clearly distinguished from the other forms of tissue which occur in the cortex; they were comprehended, together with the sieve-tubes and soft bast generally, under the common name of "Vasa propria."

The true laticiferous vessels investigated by Moldenhawer were those of *Musa* and *Chelidonium*, which he found to be composed of sacs which open into one another. He erroneously supposed that this also held good of the laticiferous cells of *Asclepias fruticosa*. The anatomical investigation of plants had not then made sufficient progress to render a systematic study of development possible, and we can only wonder that Moldenhawer came so near the truth.

Among the more modern phytotomists, Unger, in 1840, maintained that laticiferous vessels are formed by the fusion of cells, but the instance he chose was unfortunately *Ficus benghalensis*, where the laticiferous tubes are now known to be inarticulated. As Unger's view was not till much later supported by any trustworthy observations, it is not surprising to find that a different theory, although wholly without foundation, was long accepted by many botanists. This view, according to which the laticiferous vessels are simply intercellular spaces which only obtain a membrane of their own in the later stages of their development, was first expressed by Schleiden,<sup>2</sup> though not with any great confidence, and afterwards, in 1846, maintained at length by an anonymous authoress.<sup>3</sup> This intercellular theory made a great impression on botanists, which is quite intelligible considering that it was then the only view supported by researches which were really diligent and careful, although wrongly interpreted. Even Mohl<sup>4</sup> received the new theory

<sup>1</sup> 'Beiträge zur Anatomie der Pflanzen,' Kiel, 1812, pp. 136, 140, 146, 151. Compare also Sachs, 'Geschichte der Botanik,' p. 305.

<sup>2</sup> 'Grundzüge der wissenschaftlichen Botanik,' 2nd edition, 1845, part I, pp. 213, 254.

<sup>3</sup> 'Botanische Zeitung,' 1846.

<sup>4</sup> See his 'Anatomy and Physiology of the Vegetable Cell,' 1852, p. 2.



with favour, and it was still defended by writers of repute as late as the year 1860.<sup>1</sup>

The investigations in question were chiefly carried out by means of longitudinal sections through the growing point, and embraced a large number of the plants which produce latex, among which the Apocynæ, Asclepiadeæ, Urticaceæ, Euphorbiaceæ, Papaveraceæ, and Cichoriaceæ were represented.

Even the case of *Chelidonium majus*, where the remains of the cross walls persist, as is well known, during the life of the plant, seems at first not to have suggested any doubts as to the correctness of the intercellular theory. The question which was chiefly regarded in the researches of this period was, whether the laticiferous vessels do or do not possess a proper membrane from their first origin. It was supposed at that time that two distinct lamellæ of cell-wall must exist between each two neighbouring cells of any tissue, and when a double wall could not be detected between a laticiferous canal and the neighbouring parenchymatous cells, it was considered a necessary inference that the former had no cell-wall of its own at all. As soon as Mohl, only a few years later, had shown that the original wall between two neighbouring cells of a tissue is regularly a single lamella, this ground for the intercellular theory disappeared of itself.

In the mean time Schacht,<sup>2</sup> in 1851, brought forward an entirely new theory, according to which the laticiferous vessels generally are "laticiferous bast-cells, which are frequently branched." He thus refused to regard these vessels as forming an independent system, and wished to include them as a subdivision in the Bast-cell group. And here it must be remembered that Schacht and some other phytotomists of that time were in the habit of giving the name Bast to cells of the most different kind, if thick-walled, long or branched, without any regard to their position, just as in more recent times Schwendener has extended the name Bast to elements of the most various kinds which agree only in contributing to the mechanical strength of the organs to which they belong. Schacht's first researches extended to Euphorbia, Hoya, Rhizophora, *Chelidonium*, *Lactuca*, &c. His view had the one advantage over the intercellular theory, that he once more recognised the cellular nature of the elements of which the

<sup>1</sup> As, for example by Henfrey, 'Micrographic Dictionary,' art. "Laticiferous Tissue," 2nd ed.

<sup>2</sup> 'Botanische Zeitung,' 1851, p. 513.

laticiferous vessels are composed. In other respects, however, his theory was directly at variance with the facts. The fact especially that all laticiferous vessels, both the articulated and the inarticulated, form continuous open tubes which may traverse the whole plant, was ignored, and, indeed, expressly denied by Schacht, who asserts that these "bast-cells" are never connected with one another. Thus, not only the development but even the anatomy was completely misunderstood.

In 1855, Unger<sup>1</sup> came forward again as the representative of the cell-fusion theory. In the work cited all laticiferous vessels are described under the heading cell-fusions. The author expresses his view as follows:—"As the result of many researches, carried out according to this method,<sup>2</sup> it has been established that the elements of the laticiferous vessels are always cells of peculiar fluid contents. These cells appear as short or long, simple or repeatedly branched cylindrical sacs, the ends and branches of which frequently inosculate, so that a system of communicating tubes proceeds from them." The mode of development is not described more in detail; Unger had probably traced it only in the case of some plants with really articulated laticiferous vessels, such as *Chelidonium* and *Sanguinaria*,<sup>3</sup> and then assumed that the results of his researches also applied to the inarticulated latex-cells, an error which is the more excusable as the distinction between the two categories of laticiferous canals had not yet been detected.

In 1856, the first minute investigations of the development of articulated laticiferous vessels were published by Schacht,<sup>4</sup> who, however, only actually traced the process in one plant, *Carica papaya*. The remarkable latex vessels of this genus form, as is well known, a much branched system, which here, unlike most other cases, is developed on the inner side of the cambium. They form concentric circles in the xylem, tracheæ and laticiferous vessels being produced alternately by the cambial layer. The cross-walls and also portions of the side-walls are, according to Schacht, soon absorbed. The main trunks of the vessels also send out numerous branches, which are sometimes extremely thin, and these can either inosculate with other

<sup>1</sup> 'Anatomie und Physiologie der Pflanzen,' p. 157.

<sup>2</sup> Maceration and boiling with potash.

<sup>3</sup> The latex-sacs of *Sanguinaria* are not in communication, and are, therefore, of course no longer reckoned among laticiferous vessels; but this was not known in Unger's time.

<sup>4</sup> 'Monatsberichte der Berliner Akademie,' 1856, 2, p. 515.

vessels or end blind. These fine ramifications, termed capillary tubes by Schacht, are said to traverse the inter-cellular spaces. Connecting tubes are also formed in the parenchyma of the medullary rays, which are said to arise from the fusion of cells, and to establish a communication between all the vessels. In the cortex only lateral branches from the main trunks are said to occur.

Schacht's observations also extended to various other plants, of which *Sonchus* has most interest for us. In this case Schacht has not much to say about the actual development. His words are,<sup>1</sup> "The process of the fusion of cells to form the laticiferous vessels seems to be the same here as in *Carica*, with the one difference that the parenchyma cells of the medullary rays take part in the process much more rarely here than in the other case," &c. It is scarcely to be inferred from this that Schacht had really traced the development, nor do the figures which he gives<sup>2</sup> show anything of the origin from cells. On the other hand, the mode of lateral union between the vessels is correctly represented. They here send out lateral protrusions which meet those of other vessels, and establish a connection in a way similar to the process of conjugation in *Spirogyra*, &c.

There can be no doubt that this work of Schacht's marks an important advance in our knowledge of these organs. Schacht himself still struggled to save his Bast-cell theory, asserting that true bast-cells also undergo fusion,<sup>3</sup> but his position could no longer be regarded as tenable.

During the next few years comparatively little of interest was published on the laticiferous organs. In 1862 Hartig<sup>4</sup> recognised for the first time the distinction between articulated and inarticulated laticiferous vessels. Among the former he mentions the *Cichoriaceæ* and *Papaveraceæ*, as well as the *Acerineæ*, which are no longer considered as possessing true laticiferous vessels. Those of the *Euphorbiaceæ* are described as inarticulated. The distinction thus rightly introduced was not generally accepted till much later.

In the following year (1863) the knowledge of our subject was further extended by the publication of Vogl's<sup>5</sup> researches on the laticiferous vessels of two plants belonging to the *Cichoriaceæ*. He observed the development of

<sup>1</sup> Loc. cit., p. 524.

<sup>2</sup> Loc. cit., figs. 11—13.

<sup>3</sup> Loc. cit., p. 530.

<sup>4</sup> 'Botan. Zeitung,' 1862, p. 97.

<sup>5</sup> 'Sitzungsberichte der Wiener Akademie,' vol. 43 B, p. 668.



these vessels in the cambium of *Taraxacum officinale* and *Podospermum*. He gives figures of stages in which the cross-walls are still present, and also makes the interesting statement that the communication between the vessels by means of lateral branches is established before the cross-walls are absorbed. He sums up his results in the following words:—"The laticiferous vessels of both plants arise from the fusion of cambiform or sieve-cells,<sup>1</sup> which are situated one over the other or side by side; the fusion is determined by the conversion of the membranes of the cells undergoing amalgamation into pectose." Vogl, as shown by his fig. 4, plate ii, confused the young laticiferous vessels with sieve-tubes.

The statement of Trécul (to whose researches I shall return below) that a communication exists between the laticiferous vessels and the tracheæ called forth a renewed investigation of these tissue forms. Hanstein's prize essay<sup>2</sup> (1864) contains a very full treatment of the subject, in which, however, the distribution and other anatomical conditions are chiefly regarded. As regards the development, Hanstein was an unconditional supporter of the theory of cell-fusion. But he also failed to observe directly the successive stages of development. With special reference to the laticiferous vessels of the Cichoriaceæ, Campanulaceæ, and Lobeliaceæ, he says that "the fusion of these cells generally takes place at such an early stage that, owing to the delicacy of the parts which still prevails, it escapes direct observation."<sup>3</sup> He adds, however: "The arrangement of the sac-shaped trunks of the vessels corresponds exactly to that of the neighbouring cells, while the length of the latter can often be recognised in the still distinguishable joints of the sacs." A little further on he gives the grounds of his opinion still more clearly. He says:<sup>4</sup> "On the other hand, the origin of these vessels from chains of cells finds its repeated proof, on the one side, in the comparison with those of *Carica*, and on the other in the *Papaveraceæ*," &c. We thus see that Hanstein's view, like that of Unger, is supported rather by analogy than by direct observation. The argument from analogy had become, of course, much stronger since the publication of Schacht's observations on *Carica*. How incomplete the researches

<sup>1</sup> Vogl also uses the term "Leitzellen."

<sup>2</sup> 'Die Milchsaftegefäße und verwandten Organe der Rinde,' Berlin, 1864.

<sup>3</sup> Loc. cit., p. 14.

<sup>4</sup> Loc. cit., p. 15.



were on which the theory of cell-fusion was at this time based is manifest from the fact that Hanstein still extends this theory to the inarticulated latex-cells.

Dippel's work<sup>1</sup> (1865), which also received a prize from the Paris Academy, agrees with Hanstein's as regards the main results. He finds that all laticiferous vessels consist at first of cells arranged in rows, and those which do not form a network are said by him to admit of being broken up into their elements by means of maceration. Dippel, not content with simply extending these results to the inarticulated latex-tubes, claims to have actually seen the cross-walls in these also. This statement has been refuted by De Bary<sup>2</sup> and Schmalhausen, who have shown that the cross-walls seen by him did not belong to the laticiferous cells at all, but to other cells lying above or below them. The same observers have also proved the incorrectness of his conception of these vessels as modified sieve-tubes.

Trecul published, between the years 1857 and 1868, a long series of papers on the laticiferous vessels and allied organs, which are remarkable for the great number of species investigated. I will pass over his theory of circulation, according to which the laticiferous vessels correspond to the veins and the tracheæ to the arteries of animals, and only touch slightly on a few points, which are of interest with reference to the development.

Among the Papaveraceæ Trecul<sup>3</sup> observed the origin of these vessels from cells in *Argemone*. Here, as in other cases, the latex is said to be formed before the cross-walls are absorbed. And here, too, communication exists between neighbouring vessels by means of protrusions, as is the case with so many of the articulated latex vessels.

Among the Cichoriaceæ<sup>4</sup> the development is described as follows:—The laticiferous vessels arise from cells which are fused into continuous tubes. The latter are all in communication with one another, so as to form a network. The communication between the cells comes about in three different ways—1. By the fusion of cells which stand one above the other. 2. By the more or less frequent absorption of the side walls at the points where two cells or vessels are in immediate contact. 3. When the vessels are at a distance

<sup>1</sup> As the work itself was not at my disposal, I had to use De Bary's report in the '*Bot. Zeitung*,' 1867, p. 333, as well as references in the '*Vergleichende Anatomie*,' &c.

<sup>2</sup> '*Vergleichende Anatomie*,' p. 205.

<sup>3</sup> '*Comptes Rendus*,' t. 60, 1865, p. 522.

<sup>4</sup> *Ibid.*, t. 61, 1865, p. 787.

each sends out branches towards the other, which arise as protrusions. They grow between or even through the cells of the intermediate tissue until they meet and inosculate. In *Tragopogon pratensis* Trécul observed branches of this kind, which attained a length of 1.15 mm.

Among the Aroideæ<sup>1</sup> Trécul seems to have observed both the absorption of the cross-walls and the communication by means of lateral branches in various species of *Caladium*.

In another passage<sup>2</sup> Trécul calls attention to the fact that in the Euphorbiæ no trace of the origin from cells is to be perceived, and is thus the first after Hartig to observe the distinction between the articulated and inarticulated vessels.

More detailed accounts of the development are not to be found in Trécul's treatises.

The next work of importance on this subject, that by David (1872), is occupied with the inarticulated latex-cells. To him belongs the credit of finally establishing the distinction between the two classes of laticiferous organs, although he failed to discover the true mode of development of those which are inarticulated. He supposed that new latex-cells are constantly being formed in the meristem of the growing point, and, consequently, that a great number of these cells, which have been developed successively, exist in the mature plant. It is now known that the latex-cells are formed, once for all, in the embryo, and in very small numbers (in some cases six), and that the whole laticiferous system of the plant consists exclusively of the abundant ramifications of these few original cells. This discovery was first made by Schmalhausen<sup>3</sup> (1877), and afterwards confirmed by the researches of Weber in the Würzburg Laboratory.

Schmalhausen's work is also of great value as regards the development of the articulated laticiferous vessels. His observations on the mode of origin of these organs in the Cichoriaceæ seem to me to be by far the most accurate that we possess. In describing my own observations I shall often have occasion to refer to his statements, so that only his main results need be mentioned here. Schmalhausen investigated the embryos of *Tragopogon* and *Scorzonera*. He found that the rudiments of the laticiferous vessels exist in the embryo of the ripe seed, and that they are converted

<sup>1</sup> 'Comptes Rendus,' t. 61, p. 1163.

<sup>2</sup> Ibid., t. 60, p. 1349.

<sup>3</sup> 'Beiträge zur Kenntniss der Milchsafthälter der Pflanzen,' St. Petersburg, 1877.

into actual vessels during germination by means of the absorption of the cross-walls and portions of the side-walls. This process is said to begin at the root end and thence to advance to the opposite end of the embryo. The distribution of the laticiferous organs in the embryo resembles that among the Euphorbiaceæ. In both cases they form two systems, one of which belongs to the Periblem the other to the Plerome. The laticiferous vessels have nothing in common with the sieve-tubes either as regards development or structure. I reserve details for the second part of the paper.

The 'Vergleichende Anatomie' of De Bary contains, in addition to valuable original observations, a summary of the results of all researches on the subject previous to 1877. This may be regarded as pretty exactly representing the present state of the question, as very little new information has been gained since that year. The conclusion at which one must arrive is that the development of the articulated latex-vessels by cell-fusion may be regarded as established with approximate certainty, but nevertheless, that the process has only been directly observed in very few cases.<sup>1</sup>

Since 1877 only one work has appeared which requires to be noticed here. This is E. Faivre's<sup>2</sup> investigation of *Tragopogon porrifolius*. The author asserts that the ripe embryo of this plant consists essentially of parenchyma containing abundant protoplasm, neither tracheæ nor laticiferous vessels being as yet developed. The former are said to show themselves first, but even they only after the root has protruded from the envelopes of the seed. The laticiferous vessels he finds to be developed by the fusion of rows of cells, and this mode of origin could often be detected subsequently by means of the evident articulations. The latex-vessels were found in the greatest numbers in the cotyledons. Faivre also has observed the two ways in which neighbouring vessels are connected, by the fusion of transverse rows of cells, and by outgrowths. As regards the distribution, Faivre finds that an intimate relation exists between the tracheæ and the laticiferous vessels, although they are never in communication. The latex itself is said not to appear until the root has attained a length of several millimètres. I shall soon have an opportunity of showing how far I have been able to confirm Faivre's statements by my own observation.

The investigations which I am now about to describe

<sup>1</sup> See De Bary, loc. cit., pp. 199 and 203.

<sup>2</sup> 'Comptes Rendus,' t. 88, 1879, p. 269.



were undertaken at the suggestion of Professor Sachs, with the object of investigating the development of the laticiferous vessels in certain instances somewhat more closely, and as far as possible by direct observation. I have endeavoured to avoid mere conclusions from analogy with reference to the development, such as are so frequent in most of the former investigations, except those of Schmalhausen and De Bary, and to rely only on successful thin sections. The following statement, therefore, refer exclusively to quite clear and unmistakeable objects, such as only thin sections can afford.

## 2. *Original Observations.*

I will first describe the development of the laticiferous vessels in the germ plant of *Tragopogon eriospermus*, as I have investigated this object more minutely than any other, and germ plants generally have hitherto been examined by very few observers with reference to this question. It will first be necessary to describe shortly the distribution of these vessels in the seedling.

In the root of the seedling there are two distinct systems of laticiferous vessels. First, scattered and not very numerous vessels traverse the cortical parenchyma. They lie pretty close to the surface, commonly in the fourth or fifth layer of cells. Secondly, the axial cylinder possesses a number of latex-vessels, which belong to the phloem, and, as corresponds to the diarchic structure of the root, form two large opposite groups, each of which occupies about a quarter of the circumference. In the hypocotyl the laticiferous vessels preserve the same general arrangement (fig. 1). Here, however, the hypodermal vessels are much more numerous than in the root. They lie still closer under the epidermis, from which they are frequently separated only by a single layer of cells. These vessels may be either isolated or lie two or more together. The latex-vessels of the fibro-vascular system assume a distribution corresponding to the stem structure. Here, namely, they lie chiefly on the outer (phloem) side of each bundle, though they also occur in the interfascicular tissue.

In the node the fibro-vascular bundles and the latex-vessels belonging to them bend outwards into the cotyledon so that they come to lie nearer the surface in the cotyledonary sheath than in the hypocotyl. The hypodermal vessels, on the contrary, preserve a direct course in entering the cotyledons. At the point where the fibro-vascular



bundles enter the cotyledonary sheath their latex-vessels are invariably connected with the hypodermal vessels by means of cross branches. Similar connections occur repeatedly in other parts of the sheath. In the cotyledons the latex-vessels usually accompany the fibro-vascular bundles, which are here much ramified, though here also isolated latex-vessels occur. I have never been able to trace these vessels into the growing point of the germ-stem above the youngest leaves, but have always found them continued into the young leaves themselves. It appears, therefore, that the laticiferous vessels, like the fibro-vascular bundles, are never cauline (stammeigene in Nägeli's sense).

The arrangement just described exists very evidently in germ plants with the root protruding about five millimètres from the seminal envelopes. The first question to be settled is at what stage the differentiation of the organs in question first begins. With reference to this point I have investigated the like embryo in the dry seed. In a cross-section (through the cotyledonary sheath, for example) (fig. 2), in the position where the hypodermal vessels are always found at a later stage, cells are here and there to be detected which are immediately distinguishable from their neighbours by their smaller size. In the radial direction they are only half as wide as the ordinary parenchyma cells. Seen in cross-section they always appear two together, and are commonly separated from the epidermis by a single layer of cells. In a longitudinal section (fig. 3) we find that these cells form longitudinal rows, and have evidently been formed by the division of cells of the third layer from the surface by means of tangential walls. It is the outer of these two narrower rows of cells from which the hypodermal latex-vessels are developed. Even at this early stage a differentiation of the cell-contents appeared to me to be perceptible, as the cells in question contain only a few small aleurone grains, while the latter are present in great abundance in the parenchyma cells.<sup>1</sup>

The rudiments of the fibro-vascular bundles also exist in the embryo. Here, however, I was not able to distinguish the latex-vessels in their earliest stage of development from the other cells of the procambium.<sup>2</sup> It was for this reason that, in studying the first origin of the former, I turned my atten-

<sup>1</sup> This deficiency in aleurone grains extends more or less to the sister-cells of the rudimentary latex-vessels. Where this is the case it is possible that two latex-vessels were to be formed side by side, which is not at all unusual.

<sup>2</sup> Cf. Schmalhausen, loc. cit., p. 23.

tion to the hypodermal vessels, for as regards these no confusion is possible.

I have not succeeded in every case in demonstrating the presence of rudimentary laticiferous vessels in the embryo of the ripe seed. The stage of development at which the embryo enters upon its resting state in the dry seed is known to be more or less accidental. There are probably individual ripe seeds in which the cell-divisions in question have not yet taken place, and only occur at the commencement of germination. On the other hand, the rudimentary fibro-vascular bundles can invariably be distinguished with certainty. It follows that Faivre's assertions, according to which the ripe embryo consists essentially of parenchyma, in which neither tracheæ nor laticiferous vessels are "developed," give a very inaccurate representation of the real state of the case. On the other hand, Schmalhausen's results agree fully with my own.

The latex-vessels in process of formation are much easier to distinguish as soon as germination has once begun. In seeds which had lain twenty-four hours in earth, but showed no external signs of germination, I found the rudimentary hypodermal vessels pretty clearly differentiated (fig. 4). They appear as longitudinal rows of somewhat elongated cells, which are as a rule about half as wide and double as long as the parenchyma cells. It is still easy to see that they have been formed by the division of cells belonging to the third, and in some cases to the fourth layer from the surface. The sister cells, which lie on the inside, remain smaller than the other parenchyma cells, and have evidently undergone further cross-divisions, which are passed over in the cells of the laticiferous vessels. As regards the cell-contents, the differentiation is now very conspicuous. Both the epidermis and the parenchyma cells still contain a quantity of aleurone grains. The latter are entirely absent from the young laticiferous vessels. These have uniform finely granular contents, with a darker spot, which may possibly indicate the position of the nucleus.

In germinating seeds with the root just beginning to show itself, the hypodermal laticiferous vessels are a little further developed. Their contents now begin to assume the characteristic appearance of latex, and can be distinguished at once by their colour from those of all the other cells. The cross-walls, however, are still completely preserved.

In seedlings with the root protruding three to four mm. from the seminal envelopes, the laticiferous vessels can be clearly recognised in almost every part. It is at about this time

that the first indications of the absorption of the cross-walls are usually to be observed. At this stage of development the condition of these vessels is as follows :

In the root the hypodermal vessels are already in a fairly advanced state ; they contain latex, and here and there it can be seen that the cross-walls are perforated, at any rate in the middle. The other system, that which belongs to the axial cylinder, is more backward in its development. The contents of the cells are not visibly different from those of the other procambial cells ; and all their walls are still unaltered.

The same conditions prevail to a certain extent in the hypocotyl. Here, however, the development of the two systems is more nearly equal.

At the base of the cotyledons the connecting branches between the axial and the hypodermal latex-vessels are as far advanced as the main trunks themselves. They already contain some latex. In this region one occasionally finds cross-walls which are already perforated. In the upper part of the cotyledons the development is not so far advanced. The cross-walls are everywhere present, though sometimes slightly swollen. Towards the apex of the cotyledons no latex can be detected in the vessels.

The laticiferous vessels in the cotyledons already form a complex network. The connection between the main trunk is always formed by cross rows of cells, which afterwards undergo fusion. In these germ plants I have never observed union by conjugating outgrowths, which is so common elsewhere.

Cases not unfrequently occur in which one or more cells of a row are divided into two by longitudinal walls. Both the cells so formed take part in the formation of the vessel. Here a subsequent bi-partition has evidently taken place in some of the cells which already constitute the young latex-vessel, while the other cells remain undivided as usual.

In germ plants, where the root has attained a length of about six mm., all stages in the development of the laticiferous vessels can be observed. I will follow the same order as before, and begin with the hypodermal vessels of the root. These are now almost in the mature state. The cross-walls are all perforated, and it is sometimes difficult to find any traces of them. The articulations are very long, generally twice as long as the parenchyma cells, which are themselves much elongated. Thus the same relative length is still maintained as exists in the earliest stages of development. The laticiferous vessels of the axial cylinder, on the



other hand, are by no means so far advanced. The absorption of the cross-walls is here only beginning, and some of them are still perfect. The side-walls also are still without perforation.

In the hypocotyl the hypodermal vessels are just as far advanced as in the root. The cross-walls are absorbed, and a vessel may often be traced a considerable distance without finding any signs of them. The latex-vessels of the fibro-vascular bundles are further advanced than the corresponding ones in the root, but not so far as the hypodermal vessels. The cross-walls are perforated, and much larger openings are present in the side-walls. The latter fact, which I have often observed, agrees with Smalhausen's statement, that the absorption of the side-walls precedes that of the cross-walls, though other observations lead me to doubt whether this is an invariable rule.

In the cotyledons the perforations are smaller than in the hypocotyl. Here all the laticiferous vessels, whether belonging to the fibro-vascular system or not, show the same stage of development. Their articulations are comparatively short, corresponding to the slow growth of this part.

The absorption of the cross-walls in the cotyledons continues to make but slow progress. Even in seedlings, with the root about one cm. in length, the process is by no means completed (fig. 5). When two vessels are in contact large perforations already exist in the side-walls. The cross-walls, however, are only partly absorbed, and in some places the openings are still quite small.

As regards the order in which these vessels are developed in the different parts of the seedling, it appears to me that two rules are followed. In the first place the principle holds good that the latex-vessels reach maturity soonest in the parts where growth first takes place. Accordingly the hypodermal vessels of the root are completed very early, because the base of the root is the part which has the most energetic growth when germination begins. The corresponding vessels in the hypocotyl are little if at all more backward, for here also elongation begins very early. On the other hand, the cotyledons scarcely grow at all for a long time, and here too the development of the laticiferous vessels goes on very slowly, and is not completed till very late.

As mentioned above, Schmalhausen called attention to these facts. But, secondly, I have often had occasion to observe that the latex-vessels of the pterome are very backward in their development compared to the hypodermal vessels. This is particularly conspicuous in the root, but



also holds good of the hypocotyl — at any rate in its lower part. The hypodermal vessels contain latex when no signs of it are to be found in those of the plerome. And, further, the fusion of the cells is far advanced in the former at a time when it has scarcely begun in the latter. This distinction, however, does not hold good in the cotyledons. Here the development is uniform at all parts of the same cross-section.

With reference to the actual process of cell fusion I have obtained the following results:—The wall which is to be absorbed first appears somewhat swollen; the swelling, however, is not very marked. Then the membrane begins to dissolve gradually at some one point. Probably a middle lamella is present which resists solution longest, for stages are often found where the perforation is still closed by an extremely thin membrane. The opening is at first very small; it often, but by no means always, occupies the centre of the cross-wall. It increases gradually in size, and before the cross-wall has quite disappeared the contents of the two cells become continuous. As regards the lateral perforations I have only to confirm Schmalhausen's statement, that they are habitually formed in the neighbourhood of a cross-wall.

The observations on *Tragopogon* which I have just described were partly repeated on *Scorzonera hispanica*.

Here also the rudiments of the laticiferous vessels can be recognised in the ripe seed. The process of their development is essentially the same as in *Tragopogon*. Even before the cross-walls are absorbed the cells become distinguishable from the others by their contents, and no doubt already contain latex. The distribution is somewhat different in the two plants. In the seedling of *Scorzonera* almost all the laticiferous vessels belong to the phloem part of the fibro-vascular bundle, only in the cotyledons some are found isolated in the cortical parenchyma near the epidermis. In one other point *Scorzonera* differs from *Tragopogon*; in the former the outgrowths from the latex vessels, which afterwards establish communication between the main trunks, are formed at an early stage of germination. We have already seen that these outgrowths appear to be wanting in the seedling of *Tragopogon*. This mode of connection between the latex-vessels is best observed in the neighbourhood of the growing point of the stem in older plants of *Scorzonera*, and especially in their young leaves. Here it is easy to find vessels in which the articulations are still clearly distinguishable (fig. 8). These young vessels send out

lateral protrusions, which penetrate between the parenchyma cells. In a great many cases they end blind. The points at which they are formed appear to be determined by the pressure due to the turgescence of the surrounding cells, that is to say, they are formed where the resistance to their growth is least.

When the branches formed in this way happen to meet the branches or trunks of other latex-vessels their walls are absorbed at the point of contact, and their contents become continuous. The appearance presented recalls the phenomena of conjugation among the algæ—a comparison which was already made by Schacht. An important difference between the two processes is, however, to be found in the fact that a laticiferous vessel produces these outgrowths quite independently of the presence of a neighbouring vessel.

The development of the laticiferous vessels in the embryonal tissue of older plants agrees, so far as my observations extend, with that in the seedling. The vessels very generally have a crooked course, which is partly explained by the fact that the cells which take part in the formation of one and the same vessel do not necessarily lie in the same longitudinal row. It is quite usual for cells situated in contiguous rows, and at somewhat different levels, to undergo fusion by absorption of that part of the side-wall which is common to both. In this case parts of the latex-vessel will lie in the same straight line with cells which belong to the parenchyma, the vessel itself having a broken course.

With reference to the subject of this paper, I further examined the secondary cortex of *Scorzonera*, *Taraxacum*, and *Chelidonium*. In the root of *Scorzonera* laticiferous vessels are formed from the cambium in great abundance. In cross-section they appear ranged in radial rows, which are not usually separated by more than six layers of parenchyma cells. As a rule each radial row is double. The latex-vessels are accompanied by sieve-tubes, which are fairly numerous. In a tangential section it can be seen that the portions of tissue containing the latex-vessels and sieve-tubes form a coarse network, the meshes being occupied by the cortical rays. Frequent anastomoses between the vessels occur, both in the radial and tangential direction, some being direct, while others are effected by means of outgrowths. The cross-walls are absorbed very early. It is only quite near to the true cambial layer that one finds them still present. The early stages of development are

just like those in the germ plant, except that the young latex-vessels formed from the cambium are rather larger.

The laticiferous vessels of the secondary cortex are also very well developed in the root of *Taraxacum officinale*. Looked at in cross-section they are ranged in a number of concentric circles, which alternate with thick layers of parenchyma, and are broken by the cortical rays. Within each of these circular bands the latex-vessels form groups of indefinite size. They are very narrow, and have a rather crooked course. They are accompanied by small sieve-tubes. The cells from which the latex-vessels are developed have wedge-shaped ends, with the surfaces inclined to the radial plane. Accordingly the perforations are best seen in tangential section. The form and course of the vessels correspond exactly with those of the cells from which they are produced. In successful preparations one can establish that in the immediate neighbourhood of the cambium the cross-walls are still present.

In *Chelidonium majus* the origin of the laticiferous vessels from cells is so unmistakeable, even in the mature state, that an investigation of the development seems, from this point of view, superfluous. A few points, however, came before me in the course of my observations, which it may be worth while to mention.

In the secondary cortex of old roots the very numerous latex-vessels are irregularly distributed in the parenchyma. Sometimes they are isolated, sometimes two or three are in contact. Sieve-tubes are also present, and these, too, are here and there in actual contact with the laticiferous vessels. In the root the articulations are very short, about three times as long as they are broad. In the stem and leaves they are very long. Neither outgrowths nor other ramifications occur. Where, however, two vessels are in contact, the side-walls are often perforated. In the cross-walls the perforations generally remain very small, so small that it often requires careful focussing to detect them. Accordingly the contents of two successive articulations are not in very close connection. In alcohol material the continuity is generally already interrupted owing to contraction; and on solution of the cell-walls with strong sulphuric acid the contents do not remain behind as a continuous cord (as always happens in the Cichoriaceæ), but break up into a number of fragments corresponding to the constituent cells. The perforated cross-walls often have remarkable thickened ridges, with a circular section (fig. 9).

The contents of these vessels are comparatively trans-



parent, and for this reason it can here be easily demonstrated that each of the cells has a nucleus. Treated with hæmatoxylin these nuclei become just as clear as those of the parenchyma cells, which they resemble in every respect. They have a lenticular form, and are closely applied to one of the side-walls of the cell. Each has a fairly large nucleolus. The nuclei persist throughout life. I have found them in old latex-vessels which were beginning to become disorganised.

The parenchyma cells of the secondary cortex contain a quantity of compound starch grains, which are formed as soon as the cells have passed out of the cambial condition. In the young latex-vessels starch grains are entirely absent from the first, which makes it very easy to distinguish them at an early stage.

There is also one point in the distribution to which I will call attention. I have often convinced myself that laticiferous vessels also occur in the xylem of the root, and that not only in the medullary rays, but actually among the tracheæ. This is not invariably the case, but it is very frequent. I must therefore express my disagreement with Hanstein's assertion that in the *Papaveraceæ* "no true laticiferous vessels penetrate between the crowded cells and vessels of the xylem bundle."<sup>1</sup> Hanstein was of the opinion that the pitted vessels which are often found filled with latex had been mistaken for true laticiferous vessels. I have myself often seen pitted vessels in this condition. Their strongly thickened pitted walls bear no resemblance whatever to those of the real latex-vessels. In size and other characters they are also entirely different, so that confusion could hardly take place between them, even if the contents were "strongly coloured," which in the vessels in question was not the case. The latex-vessels are always much less numerous in the xylem than in the phloem. Sometimes, but extremely rarely, they are in immediate contact with the tracheæ.

The results of my very fragmentary observations may be summed up as follows :

1. It has been shown by direct observation that the laticiferous vessels of the plants investigated arise from rows of cells, of which the cross-walls, and, where two vessels are in contact, parts of the side-walls are gradually absorbed. The absorption takes place, as a rule, very early—in the seedling, for example, during the first stages of germination ;

<sup>1</sup> Loc. cit., p. 77.



in the secondary cortex shortly after the cells are severed from the cambial layer.

2. The communication between vessels not in contact is effected in two ways; partly by means of cross rows of cells which undergo fusion, and partly by means of inosculating outgrowths, which form connecting canals, as in the *Conjugatæ*.

3. Even before the cross-walls are absorbed the cells are distinguishable by their contents, and probably already contain latex.

In conclusion, I must express my hearty thanks to Prof. Sachs for the constant aid which he has given me throughout my work.

*On the LYMPHATIC SYSTEM and the MINUTE STRUCTURE of the SALIVARY GLANDS and PANCREAS.* By E. KLEIN, M.D., F.R.S.<sup>1</sup> With Plates XI and XII.

THE research, of which I propose to give an account in the following, had for its object to investigate, in continuation of my work on the lymphatic system of the skin and mucous membrane (see the 'Report of the Medical Officer of the Local Government Board' for 1879), the lymphatics of the salivary glands, and the pancreas. At the outset of this investigation I found it necessary to ascertain the exact nature and arrangement of the connective tissue, so intimately connected with the lymphatics, in the above organs, and at the same time I became convinced that the current descriptions (Henle, Heidenhain, Lavdowsky, Bermann, and others) given of the structure of the salivary glands admit in many respects of alterations and additions. I shall therefore have to record the results of these observations on the anatomy of the salivary glands and pancreas under the following three headings:—(A) the connective tissue; (B) the lymphatic system; and (C) observations on the minute structure.

The organs were obtained of man, ape, dog, rabbit, and guinea-pig; the glands were cut out as soon as practicable after death, and were hardened in spirit or in chromic acid or in a mixture of both. Sections were then prepared in the usual way, or minute bits were teased out and mounted. This latter process was especially resorted to for the study of the connective tissue, both of the glands in the fresh and hardened state. For the demonstration of the lymphatics injection by puncture (see my former Report) of a 2 per cent. solution of Brücke's Berlin blue was practised. I did not use any other injection material, *e.g.* asphalt in benzole, because I completely succeeded with the Berlin blue, this reagent being at the same time the best for the manipulation and the subsequent preparation of the respective organs.

A.—*The Connective Tissue.*

Henle<sup>2</sup> mentions that fine bundles of connective tissue pass from the interlobular parts into the lobules between the alveoli, forming a reticulated framework for these latter. Numerous lymph-corpuscles are here met with.

<sup>1</sup> Reprinted from the 'Reports of the Medical Officer of the Local Government Board,' Appendix B, 1880.

<sup>2</sup> 'Anatomie d. Menschen,' 2 Band, 1, p. 65.

Heidenhain<sup>1</sup> says of the interalveolar and interlobular connective tissue of the salivary glands that it does not present any features of special interest. In the connective tissue between the acini he finds sometimes few, sometimes numerous cells, which are either lymph-corpuscles or the large granular cells, called Waldeyer's plasma-cells.

Boll<sup>2</sup> finds between the alveoli, besides the branched cells forming the membrana propria of the alveoli, broader or narrower fibres and stellate cells. In the interstices of the alveoli, especially of stimulated glands, occur numerous lymph-corpuscles. He did not succeed in identifying these as emigrated white blood-corpuscles.

Lavdowsky<sup>3</sup> confirms Heidenhain's<sup>4</sup> and Boll's assertions as to the presence of lymph-corpuscles in the tissue between the alveoli. In the true salivary glands (the serous or albuminous glands of Heidenhain) he misses them; in the mucous glands, *e.g.* the orbital gland of the dog, most of them are identical with Waldeyer's plasma-cells. In the sublingual gland they, viz. the plasma cells, are so numerous that they form continuous streaks between the alveoli. Lavdowsky confirms Boll in saying that the ordinary small lymph-corpuscles are most numerous in stimulated glands. I shall have to correct this statement below about the plasma-cells.

The results of my own observations are these: the amount of the connective tissue separating the lobes or the groups of lobules, and further the individual lobules, and supporting the interlobular ducts and vessels, is subject to considerable variations in the different glands, but in all instances it, viz. the interlobular connective tissue, is proportionate to that penetrating into the interior of the lobules, *i.e.* with the chief duct and vessels. While in the submaxillary gland of man, and in the parotid of the ape, but especially in the parotid of the dog, most of the lobules are surrounded by a well-developed capsule of connective tissue, those of the other salivary glands are separated in many places only by delicate trabeculae of fibrous tissue. In a section through the hardened parotid of the dog, the submaxillary of man or the ape, the connective tissue around the large ducts and vessels appears of the same nature as in other localities and organs where fibrous tissue is arranged in continuous and compact masses, *i.e.* groups of bundles or tra-

<sup>1</sup> Hermann's 'Handbuch d. Physiologie,' v., p. 29.

<sup>2</sup> "Die Binde-substanz d. Drüsen," 'Archiv f. mikr. Anatomie,' Band v, pp. 334-356.

<sup>3</sup> "Zur fein. Anat. und Physiol. d. Speicheldrüsen," 'Archiv f. mikr. Anat.,' Band 13, p. 317.

<sup>4</sup> 'Studien d. Phys. Inst. zu Breslau,' iv, 1868.

beculæ of fibrous tissue running in various directions, are seen cut under various angles. Between these groups are the interfascicular spaces more or less distinct and wide, according to the state of hardening, to the nature of the hardening reagent, and especially to the state of the tissue itself. In a gland in which cedema had been present (see Heidenhain, l. c.), even the individual bundles constituting the trabeculæ are separated from one another by a district space; the same may be demonstrated by injecting Berlin blue into the interlobular tissue, as will be described further below. In ordinary preparations hardened in chromic acid or in a mixture of chromic acid and spirit, only the spaces separating the groups of bundles or the trabeculæ are visible, those between the individual bundles cannot be made out. In preparations hardened in spirit alone, it is difficult to make out in many places even the spaces between the trabeculæ.

Making a fresh preparation by teasing out the interlobular connective tissue, we meet with the ordinary broader or narrower bundles of fine connective-tissue fibrils and a few fine elastic fibrils; these latter may be followed for a very long course, and are seen to divide, and their branchlets unite with similar ones. The ordinary oval nuclei belonging to flattened connective-tissue cells with finer filamentous or broader membranous processes are everywhere met with. As will be described in detail below, ordinary lymph-corpuscles occur in various numbers in the different glands; they are most commonly met with in the sublingualis of the guinea-pig, in the sublingualis of the dog, and in some instances very numerous also in the pancreas of the dog. They are of about the size of colourless blood-corpuscles, with one spherical nucleus, staining well in dyes; the substance is pale and delicately reticulated. Now and then we come across a large oval or irregular-shaped cell with a spherical clear nucleus and a number of bright coarse granules in the cell substance—a plasma-cell of Waldeyer or a mastzelle of Ehrlich. In preparations stained with logwood, these cells are very conspicuous, owing to their granules being stained a deep purple colour.

As I shall show further below, the different glands vary considerably in this respect, *i. e.* in some these coarsely granular plasma-cells being of frequent occurrence, in others no such cells being met with.

Making a thick section through a hardened gland, and teasing out superficially the interlobular connective tissue, we meet in some places with a section through this tissue but tilted over, and we then see that the bundles of the connective tissue are very densely placed against one another, and cross each other in many directions, the few elastic fibres and the plasma-cells.



if any, are easily perceived; the connective-tissue corpuscles are noticed by their oval nucleus.

The important fact that I wish to point out as regards the arrangement of the connective tissue surrounding the large ducts and vessels, and also separating the lobules, is this, viz. *that the trabeculae or the groups of bundles are aggregated into definite plates*, which vary in breadth and thickness. These plates may be called the "*fascicle plates*," each of them being composed of a number of fasciculi or bundles of connective-tissue fibrils. These bundles within the same plate are arranged parallel, but after a short course cross each other more or less, and having separated from one another, some sooner and others later, bend off from one plate to join another neighbouring one. In a section through the interlobular connective tissue, this arrangement of the connective-tissue fasciculi into plates is easily ascertained, and it is well shown in fig. 1. In this figure at 1 these "*fascicle plates*" are seen in section, and the fibre bundles constituting them are seen cut transversely, or obliquely, or more or less longitudinally; at 2 the bundles are seen passing from one plate to another; at 3 are indicated the ordinary flattened branched connective-tissue corpuscles. These fascicle plates can be traced in all parts of the interlobular connective tissue, and also in that immediately surrounding the intralobular chief duct, provided this tissue is not too small in amount; in the parotid of the dog and ape, and in the submaxillary of man and dog, they are easily made out. In the parotid and submaxillary of the rabbit and guinea-pig, and in the sublingual gland of these animals, the amount of the connective tissue around the chief duct within a lobule is very small; it is represented by a few delicate bundles of fibrous tissue, running parallel with the duct. But between the lobules the arrangement of the bundles into the above fascicle plates is everywhere distinct, be the amount large, as in the parotid of the dog and ape, and the submaxillary of man and dog, or be it small, as in the parotid and submaxillary of the rabbit and guinea-pig. The difference between the fascicle plates in various glands and in various places of the same gland, consists merely in their breadth and thickness; the former, *i. e.* the breadth, depends on the number of bundles placed side by side in one plate, while the latter, *i. e.* the thickness, depends on the thickness of the individual bundles and their number above one another. Passing, in the same gland, from the large and thick septa between the groups of lobules or the lobes on to the smaller and thinner septa between the individual lobules, we notice a gradual decrease of the fascicle plates, both in breadth and thickness. In the parotid of the dog and the ape, in the submaxillary of man and dog,

even between the lobules of the same group, the fascicle plates are still considerable both in thickness and breadth, while in the corresponding places of the parotid and submaxillary of the rabbit and guinea-pig, the plates are very insignificant in thickness, being represented by few very delicate bundles; the flattened connective-tissue cells are now the most conspicuous features. This is well illustrated by a comparison of the interlobular connective tissue of the parotid of the dog, as represented in fig. 1, with the interlobular tissue of the submaxillary of the guinea-pig.

Excepting the connective tissue passing into the lobule with the duct and large vessels, there is little of this tissue between the alveoli. In those cases where there is between the lobules a considerable amount of connective tissue, of course arranged as fascicle plates, there is also a correspondingly great amount of connective tissue passing into the interior of the lobules. Thus, for instance, in the parotid of the dog and ape, and in the submaxillary of man, we find delicate fascicle plates continued into the lobule both in company with the chief duct, and also derived from the interlobular septa. But in other cases, *e.g.* in the parotid and submaxillary of the rabbit and guinea-pig, only delicate connective-tissue bundles and connective-tissue cells are found in these localities. Between the alveoli we meet with the capillary blood-vessels supported by few delicate bundles of connective-tissue fibres, and numerous endotheloid cells of exactly the same nature as those described of the interlobular tissue, viz. more or less branched flattened cells, each with an oval clear nucleus containing a delicate intranuclear reticulum. When removing, by shaking, pencilling, or otherwise, the alveolar epithelium, we get in many places a clear view of the interalveolar supporting tissue. The basket reticulum of flat cells forming the membrana propria of the alveoli (Boll, Heidenhain, and others) are seen separated by the capillary blood-vessels, by the above cell plates, and here and there by a delicate fibre bundle. But these latter are in some glands, *e.g.* parotid and submaxillary of rabbit and guinea-pig, distinct only in the neighbourhood of the intralobular ducts and in the periphery of the lobule. Between the alveoli there exists in some instances more distinct than in others, a general homogeneous matrix, in which the capillary blood-vessels, the cell plates and fibre bundles are embedded. This matrix appears in hardened and stained specimens of a definite "body," being slightly stained but transparent. In the human pancreas I can distinctly make it out, and am able to ascertain that, with the capillary blood-vessels, connective-tissue cells and fibre bundles, if any, it forms the one wall for the circum-alveolar lymph

spaces (see below), while the membrana propria of the alveoli forms the other.

As mentioned above, ordinary small homogeneous looking lymph-corpuscles and plasma-cells occur in the inter- and intra-alveolar tissue. The following points are worth stating as regards their occurrence:

1. In the parotid: (*a*) in the dog, the inter- and intralobular tissue contains few plasma-cells; ordinary lymph-corpuscles occur chiefly in the immediate neighbourhood of the intralobular ducts, in some glands more numerous than in others; (*b*) in the ape, the ordinary lymph-corpuscles are more numerous met with than in the dog; they occur especially in the interlobular tissue; in some places they are infiltrating the tissue around the blood-vessels to such an extent as to form adenoid sheaths around them; within the lobules they are also present in considerable numbers; (*c*) in the rabbit there is this conspicuous feature, that small and large fat-cells are present in the intra- and interlobular tissue, both isolated and in small groups; (*d*) in the guinea-pig a few ordinary lymph-corpuscles are seen in the interlobular connective tissue.

2. In the submaxillary gland: (*a*) in the dog, we find both plasma-cells and ordinary lymph-corpuscles in the inter- and intralobular tissue; (*b*) in the human submaxillary gland, the ordinary lymph-corpuscles are of common occurrence; a few fat-cells are seen in the intralobular tissue in some glands; (*c*) in the ape, a few ordinary lymph-corpuscles are present in the interlobular tissue; (*d*) in the rabbit, considerable numbers of isolated ordinary lymph-corpuscles occur in some places both in the inter- and intralobular tissue; (*e*) in the guinea-pig the connective tissue surrounding the larger ducts is sometimes infiltrated with ordinary lymph-corpuscles.

3. In the sublingual gland: (*a*) in the dog a great many lymph-corpuscles are present in the interalveolar tissue, and also within the lobules we find the alveoli separated by streaks and rows of lymph-corpuscles. These are of various sizes, each with a spherical nucleus, staining readily in logwood; their cell-substance appears transparent, and owing to their close position the outlines of the cells are more or less angular. But where they are not closely placed side by side they appear, in shape, identical with the ordinary lymph-cells. A few plasma-cells are also to be met with in the intralobular tissue. Lavdowsky (l. c., p. 318) considers the above lymph-corpuscles identical with the interstitial parenchymatous cells of the testis (Waldeyer, Mihalovich), which he assumes to be plasma-cells. In neither of these views can I agree with him, for in the first place the interstitial cells of the testis are epithelial cells, remnants of the



Wolffian body, as I have shown in a former paper ('Quarterly Journal of Microscopical Science,' April, 1879), and in the second place the interstitial cells of the sublingualis of the dog are ordinary lymph-corpuscles differing from those of the testis both in size and appearance; (*b*) in the rabbit occur a few lymph-corpuscles in the interlobular tissue, and the wall of the chief duct contains numerous such cells; (*c*) in the guinea-pig the wall of the chief duct is likewise supplied with ordinary lymph-corpuscles. The inter- and intralobular tissue contains very numerous oval nuclei, but these are not the nuclei of lymph-corpuscles, but of endotheloid connective-tissue cells. There are present in the intralobular tissue also ordinary lymph-cells, differing in numbers in the various lobules.

4. In the pancreas: (*a*) in the dog there occur occasionally a few plasma-cells; the ordinary lymph-corpuscles, however, are more numerous; in one animal I found diffuse adenoid tissue present in considerable amount, both in the inter- and intralobular tissue; (*b*) in man the ordinary lymph-corpuscles are met with both in the inter- and intralobular tissue; (*c*) in the guinea-pig a few plasma-cells are seen in the tissue immediately surrounding the larger interlobular ducts; in the intralobular tissue a few isolated ordinary lymph-corpuscles may be occasionally met with.

#### B. *The Lymphatics.*

Gianuzzi<sup>1</sup> was the first to show that the alveoli are surrounded by lymph-spaces for the greater part of their circumference; this has been fully confirmed by Heidenhain, Boll, and others. These lymph-spaces, according to Heidenhain,<sup>2</sup> "open into larger interlobular lymph-clefts, and these communicate with the circumvascular lymph-paths surrounding the larger arteries and veins, and finally pass into the lymphatic vessels of the hilum." I have convinced myself in all the salivary glands that I have examined, viz. the parotid of the dog, ape, rabbit, and guinea-pig; the submaxillary of man, ape, dog, rabbit, and guinea-pig; the sublingual gland of the dog, rabbit, and guinea-pig; the pancreas of man, dog, and guinea-pig; in injected and uninjected specimens, in fresh and hardened œdematous and non-œdematous glands, that the above inter- or rather circum-alveolar spaces are really connected with lymphatic vessels. As has been mentioned above, the interalveolar tissue, with the capillary blood-vessels embedded in it, forms the outer, the membrana propria of the alveoli the inner, limit of these spaces. But I cannot accept the general statement of Heiden-

<sup>1</sup> 'Berichte d. Sächs. Ges. d. Wiss.,' 27th November, 1865.

<sup>2</sup> Hermann's 'Physiologie,' v, p. 29.



chain (see above) as to the other lymphatics. These are best developed in glands in which the connective tissue is present in considerable quantity, such as the parotid of the dog and ape, the submaxillary of man, ape, and also of the dog. Injecting Berlin blue into the interlobular connective tissue, the injection matter readily passes into the lymphatics, and on suitably prepared sections it can be ascertained that the lymphatics are arranged as two sets of vessels: (*a*) lymphatic vessels belonging to, or surrounding the ducts, and (*b*) lymphatic vessels belonging to, or surrounding the blood-vessels, both arteries and veins. In both sets we have to do with real lymphatic tubes, whose wall, like that of the vessels in other similar organs, is a single layer of endothelial plates of an elongated shape, and with more or less sinuous outlines. The vessels are possessed of valves and corresponding saccular dilatations; they are very irregular in thickness, for very minute tubes are seen suddenly to become distended into broad sinuses.

The vessels surrounding the ducts, as well as those surrounding the blood-vessels, form rich anastomoses, but they are chiefly extending in a direction parallel to the duct and blood-vessels. The vessels of both sets anastomose with one another. Where the amount of the connective tissue in which the ducts and large blood-vessels are embedded is great, as in the above-named glands, the double arrangement of the lymphatic vessels, viz. as those of the ducts and those of the blood-vessels, is easily ascertained; but this is not the case in those glands in which the connective tissue is scanty; even in the former glands tracing them with the duct and blood-vessels into the individual lobules, the vessels of the two sets become naturally so reduced in number and so compressed in space that this double arrangement is lost.

As regards the vessels surrounding the ducts, their arrangement and nature is easily understood on a reference to the figures. That some of them are possessed of valves there can be no doubt about, from the inspection of the respective preparations.

Amongst the lymphatics of the second set, *i.e.* the perivascular lymphatics, there are also some of them that are possessed of valves. Their relation to the blood-vessels is various; either the lymphatics follow the blood-vessels surrounding them as a plexus, or the latter, both arteries and veins, appear surrounded by a lymphatic for a greater or smaller portion of their circumference, as is shown in fig. 13; or the blood-vessel is completely ensheathed in a lymphatic. One of the most striking examples of this kind is to be found in the sublingual gland of the guinea-pig. At the side of, and close to the large nerve branch (of the

*N. lingualis*) connected with the gland there is a large lymphatic vessel running parallel with the nerve; this lymphatic contains in its interior a venous blood-vessel. Fig. 10 shows this vessel and the vein in transverse section. The wall of the lymphatic consists of the lining endothelium, outside this of a delicate intima and circular muscular media, and further, of a reticulated connective-tissue adventitia. The outer surface of the vein, *i.e.* the one facing the lumen of the lymphatic, is covered with a layer of endothelium.

Following the connective tissue accompanying the ducts and large blood-vessels into the lobules, the lymphatic tubes become of course scarcer, and more reduced in the area of their distribution. At the same time they lose the character of tubes and assume more of the nature of lymph-spaces and clefts between the connective-tissue trabeculae and the fascicle plates.

The septa between the lobules, belonging to the same group or lobe contain as a rule lymphatics of the same nature as just mentioned, that is to say, they appear more of the nature of lymph-clefts and spaces in the connective tissue such as is mentioned by Gianuzzi and Heidenhain. Sections through the parotid and submaxillary of the dog, such as represented in figs. 12 and 14, whose lymphatics had been injected, show the distribution of these vessels and spaces very well.

In the submaxillary of the dog and rabbit, and in the sublingual of the guinea-pig and rabbit, *i.e.* in those glands that contain conspicuous ganglia (see below), we notice that some of the ganglia and the afferent and efferent nerve branches are surrounded or accompanied respectively by a lymphatic vessel.

In the glands that contain a considerable amount of the interlobular connective tissue, such as the parotid of the dog and ape, the submaxillary gland of man, ape and dog, the lymphatic vessels around the ducts and around the blood-vessels bear the same intimate relation, *i.e.* that of an open communication, to the spaces existing between the fascicle plates and between the bundles connecting neighbouring plates as described above. This relation has been very minutely described and figured in my former Report (for 1879), and I need not therefore again enter into this subject.

The circumalveolar lymph-spaces which, as mentioned above, were first demonstrated by Gianuzzi, form one intercommunicating system. They anastomose with the lymphatics hitherto mentioned in two ways:—(1) *At the margin of the lobules* they open into the lymphatic vessels, or in the absence of these, into the lymphatic clefts situated in the connective tissue between the lobules; (2) *those of the more central parts of the lobule* empty

themselves into the lymphatics associated with the ducts and the larger blood-vessels of the lobule.

These relations are well illustrated in figs. 12 and 14, and it does not require any lengthy description to make them understood. In all those glands, and in all those places where the interlobular tissue is reduced to thin fascicle plates with the flattened endotheloid connective-tissue corpuscles between them, as mentioned above, the circumalveolar lymph-spaces of the periphery of the lobule open into the lymph-spaces and clefts contained between these fascicle plates. The same is also the case where the ducts and the large blood-vessels entering the lobule are accompanied by delicate trabeculæ of connective tissue, for the spaces and clefts between these are the paths by which fluid passes from the circumalveolar lymph-spaces of the central portion of the lobule into the efferent lymphatics.

### *c. Observations on the Minute Structure.*

The facts which I wish to describe in this section refer to some points in the structure of the ducts and alveoli in the different salivary glands, which have not been sufficiently appreciated yet or which differ in some respects from those mentioned by other observers, notably Professor Heidenhain, in his admirable article in Hermann's 'Physiologie,' vol. v. As regards the ducts, it is necessary to consider separately the interlobular from the intralobular ducts. The first are the large ducts lined in most glands with a double layer of columnar epithelial cells. The one next to the lumen of the duct, *i.e.* the inner layer, is composed of conical or columnar cells, each with an oval nucleus in about the middle. The outer layer is composed of inverted conical cells; the basis of these cells rests on the membrana propria, while the pointed extremity is pushed in between the superficial cells. Each cell possesses in its outer part a spherical or slightly oval nucleus, which is as a rule more transparent than that of the inner layer of cells. In many instances the cell-substance of the outer and inner cells shows a more or less pronounced indication of a longitudinal fibrillation, similar to the one well known since Henle's and Pflüger's researches in the intralobular ducts (see below), or the salivary tubes of Pflüger. There are apparently great differences in this respect between the interlobular ducts of the various salivary glands. The outer layer of cells, *i.e.* the one next to the membrana propria, is not in all salivary glands equally distinct; where this layer is well represented, its nuclei contrast well with those of the inner layer by their spherical shape, small size, and great transparency.

The following is a summary of my notes on this point:



A. In the parotid :—(1) In the dog : the interlobular ducts are lined with a double layer of epithelial cells, the outer layer well developed ; no distinct fibrillation of the cell-substance. (2) In the ape : two layers of epithelial cells, the outer layer of cells not so well developed as the inner, the nuclei of the former spherical, while those of the latter are oblong. The fibrillation of the cell-substance is tolerably distinct. (3) In the guinea-pig : two layers of cells, similar to those of the parotid of the dog, only in alcohol specimens is there an indication of a fibrillar structure of the cell-substance. (4) In the rabbit : two layers of epithelial cells, the outer layer is very well developed ; traces of fibrillation of the cell-substance.

B. In the submaxillary gland :—(1) In the dog : two layers of cells, the outer layer not well developed, the nuclei of its cells spherical and small, those of the inner layer very crowded, larger, and oval. No distinct signs of fibrillation in the cell-substance. (2) In man there are two layers of cells, the outer layer well developed ; its nuclei are here larger than those of the inner layer. Distinct fibrillation of the cell-substance. (3) In the ape the relations are precisely the same as in man. In one instance I found amongst the inner layer of columnar cells some that were goblet cells. (4) In the rabbit there is an indication of an outer layer of cells only in the largest ducts, but the cell-substance, especially in its outer part, is distinctly fibrillated. (5) In the guinea-pig the interlobular ducts are very conspicuous by their wavy long course, and by their numerous branches. These latter often run side by side together for considerable distances. There is an indication of an outer layer of cells only in the larger ducts, but the fibrillation of the cell-substance is very marked.

C. In the sublingual gland :—(1) In the dog the interlobular ducts, especially in their larger branches, are lined with a double layer of columnar cells. (2) In the rabbit the chief ducts are lined with a double layer of columnar cells ; indication of fibrillation of the cell-substance. (3) In the guinea-pig the chief duct is lined with three layers of epithelial cells, an inner layer of conical or short columnar cells, a middle one of spindle-shaped cells, and an outer one of inverted conical cells. Amongst the cells of the inner layer are numerous goblet cells. The interlobular branches of the chief duct are lined with two layers of short columnar cells.

The intralobular ducts, or the salivary tubes of Pflüger, as a rule, are lined with a single layer of columnar cells, each with a spherical or slightly oval nucleus in about the middle of the cell, or even a little nearer to the inner than to the outer boundary of the epithelium. The outer part of the cell-sub-



stance, as described by Henle,<sup>1</sup> Pflüger,<sup>2</sup> and all other observers, consists of relatively coarse fibrillæ. I have shown<sup>3</sup> that these rods or fibrillæ are joined by short lateral branchlets into a reticulum. I find this reticulated arrangement of the above rods or fibrillæ specially well marked in preparations prepared in chromic acid, especially when the cells are viewed obliquely or in the bird's-eye view; with a good high magnifying power, *e.g.* Zeiss's oil  $\frac{1}{17}$ , I can make it out also in the profile view. A second or outer layer of cells is, as a rule, present only in the commencement of the intralobular ducts, and then only as composed of few cells, *i.e.* cells occurring from place to place at considerable intervals.

Distinct from these latter are the nucleated cells, being spindle-shaped or staff-shaped looking or branched, each with a flattened or angular nucleus, which are in connection with the *membrana propria*, and extend from this between the epithelial cells, in some cases even forming a sort of an inner membrane lining the lumen. This condition is well shown in some of the intralobular ducts in the parotid of the rabbit and guinea-pig.

With the cessation of the fibrillation of the outer part of the cell-substance, the intralobular duct or the salivary tube as such terminates, and its columnar epithelial cells change into polyhedral cells.

According to the accepted view, as represented by Heidenhein (Hermann's 'Physiologie,' vol. i, p. 25 and 26), the salivary tube passes into the alveoli by the intercalated or intermediate part, a fine tube, which is either lined with long spindle-shaped cells (parotid) "projecting" so far into the lumen of the alveoli, that they are surrounded by the secretory cells like the stalk by the apple (Boll, Ebner); or the intercalated part is lined with small cubical cells, which pass suddenly into the large secretory cells of the alveoli, as is the case in the submaxillary gland of the dog and rabbit (Ebner).

These statements of Heidenhain, according to my observations, require slight alterations and additions.

(1) In the parotid of those animals that I have examined I find that the salivary tube passes into the above intercalated or intermediary part through a distinct narrow short bit, which I will call the *neck*, and which is characterised by the lumen and the whole breadth of the salivary tube becoming here suddenly smaller, the lining epithelial cells more transparent, short cubical or polyhedral, and very closely placed, and showing no signs of

<sup>1</sup> 'Eingeweidelehre,' 1873.

<sup>2</sup> 'Die Endig. d. Absonderungsnerven,' &c., Bonn, 1866.

<sup>3</sup> 'Quarterly Journal of Micr. Sci.,' xix, 1879.

fibrillation of their substance. The nuclei of these cells are smaller than those of the cells of the salivary tubes, are spherical, and very conspicuously crowded, they stain very readily, and hence this part of the tube, *i.e.* the neck, is very conspicuous in preparations well stained in logwood. In the parotid of the dog and ape the neck of the salivary tubes is a very prominent feature; in that of the rabbit and guinea-pig I have not been able to make it out with sufficient clearness.

Past this *neck* and nearer to the alveolus we come to the *intercalated* or *intermediary* part of the anthers; this is a thin, long tube giving off several similar branches; they all communicate immediately with the alveoli. As regards the structure of these intercalated portions, I find that *the lumen is lined with a special delicate membrane*, commencing as such in the intercalated part; in some animals *e.g.* parotid of rabbit and guinea-pig, it is a continuation of the inner nucleated membrane lining the lumen of the salivary tube, which we mentioned as occurring in some places. *Between* this inner membrane of the intercalated part and the *membrana propria* is a *layer of flattened, long, transparent cells*, each with an oval nucleus placed parallel with the long axis of the tube. These cells are the direct continuation of the epithelium lining the salivary tube and the neck.

Such is the structure of the intercalated tubes in the parotid of the dog, ape, rabbit, and guinea-pig. In the dog and ape, the inner lining membrane is not everywhere so distinct as in the other two animals.

Now the transition of the intercalated tube into the much larger alveoli takes place in this way: the *membrana propria* of the former passes into the *membrana propria* of the latter, the flattened transparent epithelial cells of the former pass directly into the columnar or pyramidal less transparent secretory cells of the latter, while the inner lining membrane above mentioned is continued a short distance into the lumen of the alveoli, in some places still including a nucleus. It is, then, not correct to say (Boll, Ebner, Heidenhain) that the whole intercalated tube becomes surrounded by the secretory cells of the alveoli, since this is the case only with the inner lining membrane. The continuation of this membrane into the lumen of the alveoli is comparable to the centro-acinous cells of Langerhans in the pancreas, in which exactly the same relation as regards the intercalated tube and the alveoli obtains as in the parotid.

(2) In the submaxillary glands the condition of things varies greatly in the different animals. I must decidedly oppose the statement as to a uniformity existing between the intercalated tubes in the dog and rabbit, as first mentioned by Ebner and endorsed by Heidenhain. In the submaxillary of the dog I find

that the salivary tube, which is of the same structure as that in the parotid, passes through a short narrow *neck* with exactly the same distinctive characters as in the parotid, directly into the much larger alveoli, the cubical cells lining the neck directly changing into the transparent columnar mucous cells of the alveoli. In various places this neck is of different length, but according to its structure it is comparable, not to the intercalated or intermediary tube in the parotid, but to the neck of the salivary tube only. The intralobular ducts or the salivary tubes of the submaxillary of the dog would, then, differ from those of the parotid in not possessing the intermediary part at all.

A similar example, viz. the transition of the duct into the alveoli of the gland through a neck is found in the pyloric glands of the stomach.

In the serous portions (see below) of the human submaxillary gland the salivary tubes pass through a short *neck* into a long thin branched intermediary part; the structure of the neck and of the intermediary part and the transition of this latter into the alveoli, is exactly the same as in the parotid of the rabbit. As for the mucous portion of this gland, *i.e.* the human submaxillary, the condition is the same as in the submaxillary of the dog, *i.e.* the salivary tubes pass through a short *neck* directly into the alveoli. In the ape the salivary tube passes into a *neck* of much greater length than either in the dog or man. After this comes a long, thin-branched intermediary part of the same structure, and the same relation to the alveoli as in the submaxillary of man.

In the rabbit there is an indication only of a *neck*, after which follows a short and narrow intermediary part lined with flattened elongated epithelial cells, directly passing into the columnar secreting cells of the much larger alveoli.

In the guinea-pig the presence of a *neck* is not distinct; there is an intermediary part of various length and more or less branched.

(3) In the sublingual gland the conditions are these:—In the dog the intralobular duct passes through a distinct *neck* directly into the alveoli; the neck is conspicuous by its small diameter, this being smaller than either that of the duct or the alveoli; it is lined with a layer of polyhedral epithelial cells with small crowded nuclei. These cells pass directly into the columnar cells lining the alveoli.

In the sublingualis of the rabbit the conditions as regards the *neck* and the absence of the intermediary part are exactly the same as in the submaxillary gland of the dog, the two glands, viz. the sublingual gland of the rabbit and the submaxillary of the dog, being similar in structure, as will be shown below.



In the sublingualis of the guinea-pig we have to deal with a common mucous gland; hence the relation of duct and alveoli is the same as in other mucous glands *e.g.* those of the root of the tongue, palate, pharynx, and œsophagus. The small branches of the intraobular duct are lined with a single layer of polyhedral cells, which directly pass into the columnar mucous cells of the alveoli.

I have shown ('Quarterly Journal of Microscopical Science,' January 1881, p. 116) that in the guinea-pig, both in connection with the parotid and the submaxillary, there is a small flat mucous gland, *i.e.* the admaxillaris superior and inferior, the duct of each of which joins the duct of the respective salivary glands. As regards the structure of the intralobular ducts, the admaxillary glands resemble those of the submaxillary of the dog; but the alveoli of the former, *i.e.* of the admaxillary glands, are lined only and exclusively with columnar mucous cells, no real crescents being present. But there exist here pseudo-crescents (Boll), *i.e.* local thickenings of the membrana propria.

To summarise. (a) In the parotid of the dog and ape, in the serous portions of the human submaxillary gland, and in the submaxillary of the ape, the smallest branches of the salivary tubes, *i.e.* the intralobular ducts with columnar epithelial cells, whose outer portion is conspicuously fibrillated, pass through a narrow, shorter, or longer neck, with cubical epithelial cells, with small crowded nuclei deeply staining in dyes, into the intermediary or intercalated portion, a narrow, longer, or shorter branched canal and composed of an inner limiting nucleated membrane, and an outer membrana propria, and between the two a layer of transparent flattened epithelial cells with elongated nuclei. In the parotid and submaxillary of the rabbit and guinea-pig there is only an indication of the neck, but otherwise the relations are the same as before. (b) In the submaxillary gland of the dog, in the mucous portion of the submaxillary of man, in the sublingual gland of the rabbit, and in the admaxillary glands of the guinea-pig, the salivary tubes pass through the neck of the same nature as above directly into the alveoli. The intermediary part is therefore absent. (c) In the sublingual gland of the dog and guinea-pig the intralobular ducts of the same nature as in the ordinary common mucous glands, and they pass directly into the alveoli.

The alveoli of all the glands examined are tubes more or less branched and convoluted. In all those that are concerned in the secretion of mucus, *i.e.* the submaxillary gland of the dog, the mucous portion of the submaxillary of man and the ape, the sublingual of the dog, rabbit, and guinea-pig, and the admaxillary glands of the last-named animal, the tubular and branched



character of the alveoli is easily ascertained, owing to their comparatively slightly convoluted nature; in the serous or albuminous salivary glands, however, this tubular and branched character, although existing to the same extent as in the others, is nevertheless not so conspicuous, owing to the alveoli being very close and much convoluted. Hence, in a thin section the same tube will be found cut several times in succession, most of the tubes appearing cut transversely, few also obliquely. But making a thick section through a moderately hardened gland, or making a section through a fresh gland, and teasing this slightly out, the tubular, branched, and convoluted nature of the alveoli can be ascertained beyond any doubt. A representation of the shape and nature of the alveoli of the albuminous salivary glands (parotid), as given by Heidenhain in fig. 3 in the article several times quoted, is taken from a gland excessively shrunk; it does not by any means correspond to the natural state of the alveoli. The parotid of the dog, the parotid and the submaxillary of the rabbit, the submaxillary of man, when not excessively hardened by spirit, when prepared with great care in diluted alcohol (two parts of methylated alcohol and one part of water), or in the mixture of chromic acid and spirit, above mentioned, or in a  $\frac{1}{6}$  p. c. solution of chromic acid, shows the tubular, branched, and convoluted nature of the alveoli with sufficient distinctness. In all albuminous or serous salivary glands some of the alveoli are connected with one another from place to place by very thin and short bridges, composed of two or three secretory cells. These bridges appear *solid*, no lumen being perceptible in them, and it is probable that they are the means of connecting neighbouring alveoli that lead into neighbouring ducts. In the case of the muco-salivary glands I have not seen these solid bridges; here the alveoli are often more or less constricted at the point of dividing, but the lumen is always traceable through these constrictions.

As regards the membrana propria of the alveoli in the different glands, there is nothing new that I can bring forward, since this subject has been so thoroughly investigated by Boll, Heidenhain, and Lavdowsky. But as regards the lumen and the lining epithelium, the following points are worth mentioning:—The glands which I have examined can be arranged in the following groups, taking the structure of the alveoli and the intralobular ducts as the distinguishing characters. (a) Albuminous (serous) glands; the parotid of all animals; the submaxillary of the rabbit; the submaxillary of the guinea-pig; the serous (major) portion of the submaxillary gland of man and ape. (b) Compound mucous glands; as such are considered those in which the alveoli are lined with mucous cells, and *out-*

*side* these are the crescents of Gianuzzi, *e.g.* the submaxillary of the dog and the sublingualis of the rabbit. (c) Simple mucous glands; as such are considered those the alveoli of which are lined with mucous cells, but *outside* these there are no cells comparable to the cells of the "crescents." They are of two kinds. (1) The admaxillary glands (superior and inferior) of the guinea-pig, and the mucous portion of the submaxillary of man and ape; in these the ducts are similar in structure to the salivary tubes of the above glands (*i.e.*) conspicuously fibrillated in the outer part of the substance of the columnar epithelium), and the alveoli are lined with uniform typical columnar or goblet-shaped transparent mucous cells; (2) the sublingual gland of the dog and the sublingual gland of the guinea-pig: in these the intralobular ducts are, like the ducts of the common mucous glands at the root of the tongue and palate, lined with cubical cells without conspicuous fibrillation; the alveoli are lined either only with typical columnar transparent mucous cells (guinea-pig), or *with mucous cells and albuminous cells* (dog). (See below.)

(a) As regards the albuminous glands, the lumen of their alveoli is a small canal, which in the state of active secretion of the alveoli may dwindle down almost to its disappearance. The cells lining the alveoli are a single layer of columnar or pyramidal cells, whose substance is in hardened specimens a uniform, dense, honeycombed reticulum. I have preparations of the hardened resting parotid and submaxillary of the ape, and especially of the submaxillary of the rabbit, where in all alveoli the epithelial cells show a uniform beautiful reticulum, and as I have described in my paper on the structure of cells and nuclei in the April number, 1879, of the 'Quarterly Journal of Microscience.' Heidenhain (l. c., p. 18) thinks this representation diagrammatic; but against this I have this to say, that the reticulum in the cells in my preparation of the above-mentioned glands is so uniform and beautiful that it is impossible to make of it a diagrammatic representation.

In the parotid of the rabbit I have noticed in some instances that some of the alveoli are marked from the others by the presence of smaller and larger globular highly refractive granules, such as are mentioned by Langley ('Proceedings of the Royal Society,' No. 198, 1879). The granules stain slightly in hæmatoxylin, and differ in size between that of a coloured blood-corpuscle and a minute dot. Where the cells are viewed in profile, the granules are seen to occupy the inner part of the cell-substance (Langley's state of secretion) the outer part is therefore non-transparent, and here the intracellular reticulum can be made out. Also in the inner part with good illumination and high

powers it can be ascertained that this reticulum is present and that the above granules are contained *in* its meshes.

Each epithelial cell has in its outer third a spherical nucleus, which in many places, both in preparations hardened in chromic acid and in spirit, is more or less angular. But this state of the nucleus is clearly due to shrinking, because in those specimens where the shrinking is more pronounced, noticeable by the closely packed state and the smallness of the alveoli, also the angular condition of the nuclei is more striking. Such a nature of the alveoli of the cells and their nuclei, as figured by Heidenhain (l. c., fig. 3.) *i.e.* representing the alveoli of the rabbit's parotid in the resting state, cannot for a moment be admitted as typical, for it is due to an excessive shrinking of the gland by alcohol.

The submaxillary of the guinea-pig is described by Boll (l.c.) as a mixed gland, and as such is also mentioned by Heidenhain, viz. as a gland, the alveoli of which are lined either only with "albuminous" cells, or only with "mucous" cells. This I do not find to be the case, as I have already before ('Quart. Journal of Micr. Science,' January, 1881, p. 115) pointed out. I have shown that the submaxillary gland of the guinea-pig is an albuminous gland, to which, in the same manner as to the parotid of this animal, is attached a small flattened mucous gland, *i.e.* the admaxillary inferior, and to which reference has been made above.

The alveoli of the submaxillary of the guinea-pig have a very small lumen, and are lined with columnar or pyramidal cells of a very similar nature to those that line the alveoli of the pancreas, well known from the observations of Langerhans and Heidenhain, viz. the outer part of the cell-substance containing the spherical nucleus is more opaque than the inner part; in preparations stained with carmine or hæmatoxylin, the former stains readily, while the inner transparent part does not do so. In the outer part is seen in many places the same kind of longitudinal fibrillation as described and figured by Heidenhain (l. c., p. 174, figs. 43 and 44), and as is shown in fig. 6 accompanying this Report. The nucleus contains here, as well as in the pancreas, a well-developed uniform intranuclear reticulum; in some nuclei one or two large particles or nucleoli are seen connected with this reticulum. Owing to this similarity in appearance between the cells of the submaxillary of the guinea-pig and those of the pancreas, I tried to ascertain whether the former gland contains any proteolytic ferment. I have accordingly cleared it as much as possible of its connective tissue, and after having left it for several hours exposed to the air, I have subjected it to the pro-



cess of self-digestion commonly used in the study of the pancreas-ferment, *i.e.* the trypsin (Heidenhain, l. c., Foster and Langley, 'Practical Physiology'), but I have not obtained any decisive results.

In another series of experiments I have prepared a glycerin extract of the gland after the same manner as used in the case of trypsin, and have exposed in it, diluted with carbonate of sodium (l.p.c.) at 35—40 centigrades, pieces of fibrin, but have not obtained any decisive results. When, however, diluting the glycerin extract of the gland with 1 pro mille hydrochloric acid, the fibrin, after having swollen up, broke up into tiny fragments in a similar manner as is the case in pancreas digestion. On the whole, then, it seems doubtful whether the submaxillary contains trypsin, but this question would require for its definitive settlement more extended observations than I have been able to devote to it.

The great mass of the alveoli of the submaxillary of man are albuminous, of the same structure as in the rabbit; in some glands there are more, in others less numerous alveoli, which are lined with mucous cells. But in no gland do they form more than an insignificant section. The mucous cells are typical, and are the same as in other mucous glands (see below). Such alveoli are in direct continuation with the alveoli lined only with albuminous cells. As I have pointed out in a previous paper ('Quarterly Journal of Micr. Science,' April 1879), the alveoli lined with mucous cells are larger, and their lumen is larger than those lined with albuminous cells. That these mucous alveoli are directly continuous with the albuminous alveoli, of this I am fully convinced, as also of the fact that where a mucous alveolus is viewed more or less obliquely just at the point of its transition into an albuminous one, the appearance is produced as if at the cut end there were albuminous cells placed *outside* the mucous cells, and as if forming here a crescentic group similar to the crescents in the submaxillary of the dog. But the two are totally different from one another, for in the former the apparent crescents of albuminous cells are in reality the cells lining the lumen of the albuminous portion of the alveolus, continuous with the mucous portion.

In one of the two apes that I have examined on this point, I found only in the submaxillary of one indications of mucous alveoli; they were present in very small numbers amongst the great mass of albuminous alveoli, and the two were also here continuous with another.



*(b)—The Compound Mucous Glands.*

To the exhaustive description given by Heidenhain, Lavdowsky, and others of the structure of these glands, *i.e.* of the submaxillary and orbital gland of the dog, I have nothing to add. As I pointed out in a former paper ('Quarterly Journal of Micr. Science,' April, 1879) the substance of the mucous cells lining the lumen of the alveoli is a distinct reticulum, the meshes of this reticulum being occupied in the resting state by a transparent substance, the mucigen of Heidenhain; during secretion this substance changes into mucin, which in hæmatoxylin specimens is very marked by its deep purple staining. In some places the intracellular reticulum shows a different arrangement in the inner and outer portions of the cells, being longitudinal in the former and uniform in the latter, but this is not seen in all parts of the gland. And for this reason I must correct my former statement on this subject ('Quarterly Journal of Micr. Science,' 1879, p. 141) to the effect that this different arrangement of the intracellular network is not uniformly present in the whole gland.

The nucleus of the mucous cells is, *cæteris paribus*, in chromic acid specimens more compressed than in those hardened in alcohol; in the latter instance we find in many cells the nucleus spherical or slightly oval, and containing a uniform delicate reticulum. In both cases we find in many alveoli, that in some mucous cells the nucleus in its inner half has identified itself with the cell-substance, while in others it has altogether disappeared. I consider this as a very strong support to the actual and intimate connection between the intracellular and intranuclear reticulum maintained by me in the above-named paper ('Quarterly Journal of Micr. Science,' 1879). Stricker ('Vienna Sitzungsber.,' June, 1877) has shown that in some of the colourless corpuscles of the frog's blood the substance representing the nucleus is part of the cell-substance, becoming separated off from this by the appearance of the nuclear membrane, and becoming again fused with the rest of the cell-substance after the disappearance of that membrane. The appearances that I find in the nuclei of the mucous cells of the submaxillary gland of the dog, and in the mucous alveoli of the submaxillary of man, are such as to leave little doubt that such is probably also the case in these glands.

The sublingual of the rabbit, as mentioned above, is of the same structure as the submaxillary of the dog, but the alveoli of the latter are larger than those of the former; in this difference of size participate the lumen, the mucous cells, and the cells of

the crescents. The crescents are not so marked as in the submaxillary gland of the dog.

(c)—*The Simple Mucous Glands.*

As mentioned above, the sublingual gland of the dog, the sublingual of the guinea-pig, the upper and lower admaxillary of the guinea-pig, and lastly, the mucous portions of the submaxillary of man and the ape, belong to this group.

The alveoli of the sublingual gland of the guinea-pig and the two admaxillaries of the same animal are of exactly the same nature as those of the mucous glands at the root of the tongue or palate, both in the size of the alveoli as a whole and of their lumen, as well as in respect to the nature and size of the lining epithelium; this latter is a single layer of typical mucous cells. What has been said above (submaxillary of the dog) with reference to the disappearance of the nucleus in some of the epithelial cells applies also to these simple mucous glands.

In the sublingualis of the dog the relations are somewhat different. Here we find the alveoli either lined only with mucous cells or only with albuminous cells, or with both. Compare Heidenhain (l. c., p. 21) and also fig. 8 accompanying this Report. The mucous cells are columnar and slightly different from those of the submaxillary of the dog, inasmuch as there appears in most of them, in the resting gland, a trace of granular-looking protoplasm left in the outer part of the cell, *i.e.* next to the membrana propria, and in this part lies the spherical or slightly oval nucleus. The albuminous cells are also columnar or pyramidal, and their substance appears longitudinally striated owing to the intracellular network having pre-eminently a longitudinal arrangement, such as I described of the columnar epithelial cells of the intestine. Those alveoli that are lined only with albuminous cells are smaller, and their lumen is smaller than those lined with mucous cells. In many alveoli a direct transition is noticed of the mucous cells into the albuminous cells, as also the coexistence in the same alveolus of the two kinds of cells. A similar condition has been pointed out above to exist in the mucous portion of the sub-maxillary of man and the ape. Where the two kinds of cells are side by side, it is generally noticed that there are cells which, as regards appearance, stand about midway between the two; that is to say, a smaller or larger inner portion of the cell presenting the transparency of the mucous cells, while the rest consists of the more opaque substance of the albuminous cells; the greater transparency being due, as in the other mucous cells, to the distension of the meshes of the intracellular reticulum by mucigen or mucin respectively.

The albuminous cells in the sublingual gland of the dog bear,

then, a different morphological relation to the mucous cells from that existing in the submaxillary of the dog, in the latter forming crescenting groups *outside* the mucous cells. Whether in the submaxillary of the dog the albuminous cells of the crescents are under ordinary conditions destined to replace the mucous cells, these latter becoming altogether destroyed, as maintained by Heidenhain and his pupils, is a subject into which I cannot here enter. I have expressed myself against this view in my former paper ('Quarterly Journal of Micr. Science,' April, 1879), and I have seen in my specimens nothing since to make me alter this view, nor have I read in Heidenhain's latest article on this subject any new arguments which would place his assertion on a more firm basis than it stood previously.

But this I must add, that I see no reason whatever for grouping together, as Heidenhain does, the albuminous cells lining the lumen of the alveoli in the sublingualis of the dog with the albuminous cells that forms the crescents in the dog's submaxillary gland. The former, besides their different relation to the mucous cells, under ordinary conditions undoubtedly change into the latter, a fact that, to my mind, has yet to be proved for the cells of the crescents in the submaxillary of the dog.

The last subject that I wish to mention is the distribution of ganglia in the salivary glands that were examined. In the parotid in none of the above animals were there found groups of ganglion cells in connection with the nerve branches, nor isolated ones either.

In the submaxillary of the dog the ganglia are notorious, and are very conspicuous by their number and size. In the submaxillary of the rabbit and the conditions are about the same. In the submaxillary of the ape the ganglia are present, but in smaller numbers than in the preceding animals. In the human submaxillary the ganglia are insignificant, both as regards size and numbers. In the submaxillary of the guinea-pig I have not been able to find any.

The sublingualis also differs in this respect in the different animals. In the dog I miss them, while in the rabbit and guinea-pig they are very conspicuous; they are found, as shown in fig. 15, in connection with the nerve-trunks accompanying the chief duct of the gland.



A RENEWED STUDY *of the* GERMINAL LAYERS *of the* CHICK.  
By F. M. BALFOUR, LL.D., F.R.S., Trinity College, Cambridge, and F. DEIGHTON, B.A., St. Peter's College. (With Plates XIII, XIV and XV.)

THE formation of the germinal layers in the chick has been so often and so fully dealt with in recent years, that we consider some explanation to be required of the reasons which have induced us to add to the long list of memoirs on this subject. Our reasons are twofold. In the first place the principal results we have to record have already been briefly put forward in a 'Treatise on Comparative Embryology' by one of us; and it seemed desirable that the data on which the conclusions there stated rest should be recorded with greater detail than was possible in such a treatise. In the second place, our observations differ from those of most other investigators, in that they were primarily made with the object of testing a theory as to the nature of the primitive streak. As such they form a contribution to comparative embryology; since our object has been to investigate how far the phenomena of the formation of the germinal layers in the chick admit of being compared with those of lower and less modified vertebrate types.

We do not propose to weary the reader by giving a new version of the often told history of the views of various writers on the germinal layers in the chick, but our references to other investigators will be in the main confined to a comparison of our results with those of two embryologists, who have published their memoirs since our observations were made. One of them is L. Gerlach, who published a short memoir<sup>1</sup> in April last, and the other is C. Koller, who has published his memoir<sup>2</sup> still more recently. Both of them cover part of the ground of our investigations, and their results are in many, though not in all points, in harmony with our own. Both of them, moreover, lay stress on certain features in the development which have escaped our attention. We desired to work over these points again, but various circumstances have prevented our doing so, and we have accordingly thought it best to publish our observations as they stand, in spite of their incompleteness, merely indicating where the most important gaps occur.

<sup>1</sup> "Ueb. d. entodermale Entstehungswiese d. Chorda dorsalis," 'Biol. Centralblatt,' vol. i, Nos. 1 and 2.

<sup>2</sup> "Untersuch. üb. d. Blätterbildung im Hühnerkeim," 'Archiv. f. mikr. Anat.,' vol. xx, 1881.



Our observations commence at a stage a few hours after hatching, but before the appearance of the primitive streak.

The area pellucida is at this stage nearly spherical. In it there is a large oval opaque patch, which is continued to the hinder border of the area. This opaque patch has received the name of the embryonic shield—a somewhat inappropriate name, since the structure in question has no very definite connection with the formation of the embryo.

Koller describes, at this stage, in addition to the so-called embryonic shield, a sickle-shaped opaque appearance at the hinder border of the area pellucida.

We have not made any fresh investigations for the purpose of testing Koller's statements on this subject.

Embryologists are in the main agreed as to the structure of the blastoderm at this stage. There is (Pl. XIII, Ser. A, 1 and 2) the epiblast above, forming a continuous layer, extending over the whole of the area opaca and area pellucida. In the former its cells are arranged as a single row, and are cubical or slightly flattened. In the latter the cells are more columnar, and form, in the centre especially, more or less clearly, a double row; many of them, however, extend through the whole thickness of the layer.

We have obtained evidence at this stage which tends to show that at its outer border the epiblast grows not merely by the division of its own cells, but also by the addition of cells derived from the yolk below. The epiblast has been observed to extend itself over the yolk by a similar process in many invertebrate forms.

Below the epiblast there is placed, in the peripheral part of the area opaca, simply white yolk; while in a ring immediately outside and concentric with the area pellucida, there is a closely-packed layer of cells, known as the *germinal wall*. The constituent cells of this wall are in part relatively small, of a spherical shape, with a distinct nucleus, and a granular and not very abundant protoplasm; and in part large and spherical, filled up with highly refracting yolk particles of variable size, which usually render the nucleus (which is probably present) invisible (A, 1 and 2). This mass of cells rests, on its outer side, on a layer of white yolk.

The sickle-shaped structure, visible in surface veins, is stated by Koller to be due to a special thickening of the germinal wall. We have not found this to be a very distinctly marked structure in our sections.

In the region of the area pellucida there is placed below the epiblast a more or less irregular layer of cells. This layer is continuous, peripherally, with the germinal wall; and is composed of cells, which are distinguished both by their flattened

or oval shape and more granular protoplasm from the epiblast-cells above, to which, moreover, they are by no means closely attached. Amongst these cells a few larger cells are usually present, similar to those we have already described as forming an important constituent of the germinal wall.

We have figured two sections of a blastoderm of this age (Ser. A, 1 and 2) mainly to show the arrangement of these cells. A large portion of them, considerably more flattened than the remainder, form a continuous membrane over the whole of the area pellucida, except usually for a small area in front, where the membrane is more or less interrupted. This layer is the hypoblast (*hy.*). The remaining cells are interposed between this layer and the epiblast. In front of the embryonic shield there are either comparatively few or none of these cells present (Ser. A, 1), but in the region of the embryonic shield they are very numerous (Ser. A, 2), and are, without doubt, the main cause of the opacity of this part of the area pellucida. These cells may be regarded as not yet completely differentiated segmentation spheres.

In many blastoderms, not easily distinguishable in surface views from those which have the characters just described, the hypoblastic sheet is often much less completely differentiated, and we have met with other blastoderms, again, in which the hypoblastic sheet was completely established, except at the hinder part of the embryonic shield; where, in place of it and of the cells between it and the epiblast, there was only to be found a thickish layer of rounded cells, continuous behind with the germinal wall.

In the next stage, of which we have examined surface views and sections, there is already a well-formed primitive streak.

The area pellucida is still nearly spherical, the embryonic shield has either disappeared or become much less obvious, but there is present a dark linear streak, extending from the posterior border of the area pellucida towards the centre, its total length being about one third, or even less, of the diameter of the area. This streak is the *primitive streak*. It enlarges considerably behind, where it joins the germinal wall. By Koller and Gerlach it is described as joining the sickle-shaped structure already spoken of. We have in some instances found the posterior end of the primitive streak extending laterally in the form of two wings (Pl. XV, fig. 1). These extensions are, no doubt, the sickle; but the figures given by Koller appear to us somewhat diagrammatic. One or two of the figures of early primitive streaks in the sparrow, given by Kupffer and Benecke,<sup>1</sup>

<sup>1</sup> "Photogramme d. Ontogenie d. Vogel." Nova Acta. K. Leop. Carol, 'Deutschen Akad. d. Naturfor.,' Bd. x, 41, 1879.

correspond more closely with what we have found, except that in these figures the primitive streak does not reach the end of the area pellucida, which it certainly usually does at this early stage in the chick.

Sections through the area pellucida (Pl. XIII, ser. b and c) give the following results as to the structure of its constituent parts.

The epiblast cells have undergone division to a considerable extent, and in the middle part, especially, are decidedly more columnar than at an earlier stage, and distinctly divided into two rows, the nuclei of which form two more or less distinct layers.

In the region in front of the primitive streak the cells of the lower part of the blastoderm have arranged themselves as a definite layer, the cells of which are not so flat as is the case with the hypoblast cells of the posterior part of the blastoderm, and in the older specimens of this stage they are very decidedly more columnar than in the younger specimens.

The primitive streak is however the most interesting structure in the area pellucida at this stage.

The feature which most obviously strikes the observer in transverse sections through it is the fact, proved by Kölliker, that it is mainly due to a proliferation of the epiblast cells along an axial streak, which, roughly speaking, corresponds with the dark line visible in surface views. In the youngest specimens and at the front end of the primitive streak, the proliferated cells do not extend laterally beyond the region of their origin, but in the older specimens they have a considerable lateral extension.

The hypoblast can, in most instances, be traced as a distinct layer underneath the primitive streak, although it is usually less easy to follow it in that region than elsewhere, and in some cases it can hardly be distinctly separated from the superjacent cells.

The cells undoubtedly formed by a proliferation of the epiblast, form a compact mass extending downwards towards the hypoblast; but between this mass and the hypoblast there are almost always present along the whole length of the primitive streak a number of cells, more or less loosely arranged, and decidedly more granular than the proliferated cells. Amongst these loosely arranged cells there are to be found a certain number of large spherical cells filled with yolk granules. Sometimes these cells are entirely confined to the region of primitive streak, at other times they are continuous laterally with cells irregularly scattered between the hypoblast and epiblast (Ser. c, 2), which are clearly the remnants of the undifferentiated cells of the embryonic shield. The junction between these cells and the cells of the primi-



tive streak derived from the epiblast is often obscure, the two sets of cells becoming partially intermingled. The facility with which the cells we have just spoken of can be recognised varies more-over greatly in different instances. In some cases they are very obvious (Ser. c), while in other cases they can only be distinguished by a careful examination of good sections.

The cells of the primitive streak between the epiblast and the hypoblast are without doubt mesoblastic, and constitute the first portion of the mesoblast which is established. The section of these cells attached to the epiblast, in our opinion, clearly originates from the epiblast; while the looser cells adjoining the hypoblast must, it appears to us, be admitted to have their origin in the indifferent cells of the embryonic shield, placed between the epiblast and the hypoblast, and also very probably in a distinct proliferation from the hypoblast below the primitive streak.

Posteriorly the breadth of the streak of epiblast which buds off the cells of the primitive streak widens considerably, and in the case of the blastoderm with the earliest primitive streaks extends into the region of the area opaca. The widening of the primitive streak behind is shown in Ser. B, 3; Ser. c, 2; and Ser. E, 4. Where very marked it gives rise to the sickle-shaped appearance upon which so much stress has been laid by Koller and Gerlach. In the case of one of the youngest of our blastoderms of this stage in which we found in surface views (Pl. XV, fig. 1.) a very well-marked sickle-shaped appearance at the hind end of the primitive streak, the appearance was caused, as is clearly brought out by our sections, by a thickening of the hypoblast of the germinal wall.

There is a short gap in our observations between the stage with a young primitive streak and the first described stage in which no such structure is present. This gap has been filled up both by Gerlach and Koller.

Gerlach states that during this period a small portion of the epiblast, within the region of the area opaca, but close to the posterior border of the area pellucida, becomes thickened by a proliferation of its cells. This portion gradually grows outwards laterally, forming in this way a sickle-shaped structure. From the middle of this sickle a process next grows forward into the area pellucida. This process is the primitive streak, and it is formed, like the sickle, of proliferating epiblast cells.

Koller<sup>1</sup> described the sickle and the growth forwards from it of the primitive streak in surface views somewhat before Gerlach; and in his later memoir has entered with considerable detail

<sup>1</sup> "Beitr. z. Kenntniss d. Hühnerkeims im Beginne d. Bebrütung," 'Sitz. d. k. Akad. Wiss.,' iv Abth., 1879.



into the part played by the various layers in the formation of this structure.

He believes, as already mentioned, that the sickle-shaped structure, which appears according to him at an earlier stage than is admitted by Gerlach, is in the first instance due to a thickening of the hypoblast. At a later stage he finds that the epiblast in the centre of the sickle becomes thickened, and that a groove makes its appearance in this thickening which he calls the "Sichel-rinne." This groove is identical with that first described by Kupffer and Benecke<sup>1</sup> in the sparrow and fowl. We have never, however, found very clear indications of it in our sections.

In the next stage, Koller states that, in the region immediately in front of the "Sichel-rinne," a prominence appears which he calls the Sichelknopf, and from this a process grows forwards which constitutes the primitive streak. This structure is in main derived from a proliferation of epiblast cells, but Koller admits that some of the cells just above the hypoblast in the region of the Sichelknopf are probably derived from the hypoblast. Since these cells form part of the mesoblast it is obvious that Koller's views on the origin of the mesoblast of the primitive streak closely approach those which we have put forward.

The primitive streak starting, as we have seen, at the hinder border of the area pellucida, soon elongates till it eventually occupies at least two thirds of the length of the area. As Koller (*loc. cit.*) has stated this can only be supposed to happen in one of two ways, viz. either by a progression forward of the region of epiblast budding off mesoblast, or by an interstitial growth of area of budding epiblast. Koller adopts the second of these alternatives, but we cannot follow him in doing so. The simplest method of testing the point is by measuring the distance between the front end of the primitive streak and the front border of the area pellucida at different stages of growth of the primitive streak. If this distance diminishes with the elongation of the primitive streak then clearly the second of the two alternatives is out of the question.

We have made measurements to test this point, and find that the diminution of the space between the front end of the primitive streak and the anterior border of the area pellucida is very marked up to the period in which the medullary plate first becomes established. We can further point in support of our view to the fact that the extent of the growth lateralwards of the mesoblast from the sides of the primitive streak is always less in front than behind; which would seem to indicate that the front part of the streak is the part formed latest. Our view as to the

<sup>1</sup> 'Die erste Entwickl. an Eier d. Reptilien.' Königsberg, 1878.

elongation of the primitive streak appears to be that adopted by Gerlach.

Our next stage includes roughly the period commencing slightly before the first formation of a groove along the primitive streak, known as the primitive groove, and terminating immediately before the first trace of the notochord makes its appearance. After the close of the last stage the primitive streak gradually elongates, till it occupies fully two thirds of the diameter of the area pellucida. The latter structure also soon changes its form from a circular to an oval, and finally becomes pyriform with the narrow end behind, while the primitive streak occupying two thirds of its long axis becomes in most instances marked by a light linear band along the centre, which constitutes the primitive groove.

In surface views the primitive streak often appears to stop short of the hinder border of the area pellucida.

During the period in which the external changes, which we have thus briefly described, take place in the area pellucida, great modifications are effected in the characters of the germinal layers. The most important of these concern the region in front of the primitive streak; but they will be better understood if we commence our description with the changes in the primitive streak itself.

In the older embryos belonging to our last stage we pointed out that the mesoblast of the primitive streak was commencing to extend outwards from the median line in the form of two lateral sheets. This growth of the mesoblast is continued rapidly during the present stage, so that during the latter part of it any section through the primitive streak has approximately the characters of Ser. 1, 5.

The mesoblast is attached in the median line to the epiblast. Laterally it extends outwards to the edge of the area pellucida, and in older embryos may even form a thickening beyond the edge (fig. 6). Beneath the denser part of the mesoblast, and attached to the epiblast, a portion composed of stellate cells may in the majority of instances be recognised, especially in the front part of the primitive streak. We believe these stellate cells to be in the main directly derived from the more granular cells of the previous stage. The hypoblast forms a sheet of flattened cells, which can be distinctly traced for the whole breadth of the area pellucida, though closely attached to the mesoblast above.

In sections we find that the primitive streak extends back to the border of the area pellucida, and even for some distance beyond. The attachment to the epiblast is wider behind; but the thickness of the mesoblast is not usually greater in the median line than it is laterally, and for this reason probably the

posterior part of the streak fails to show up in surface views. The thinning out of the median portion of the mesoblast of the primitive streak is shown in a longitudinal section of a duck's blastoderm of this stage (fig. D). The same figure also shows that the hypoblastic sheet becomes somewhat thicker behind, and more independent of the parts above.

A careful study of the peripheral part of the area pellucida, in the region of the primitive streak, in older embryos of this stage, shows that the hypoblast is here thickened, and that its upper part, *i.e.* that adjoining the mesoblast, is often formed of stellate cells, many of which give the impression of being in the act of passing into the mesoblast above. At a later stage the mesoblast of the vascular area undoubtedly receives accessions of cells from the yolk below; so that we see no grounds for mistrusting the appearances just spoken of, or for doubting that they are to be interpreted in the sense suggested.

We have already stated that during the greater part of the present stage a groove, known as the primitive groove, is to be found along the dorsal median line of the primitive streak.

The extent to which this groove is developed appears to be subject to very great variation. On the average it is, perhaps, slightly deeper than it is represented in Ser. I, 5. In some cases it is very much deeper. One of the latter is represented in fig. G. It has here the appearance of a narrow slit, and sections of it give the impression of the mesoblast originating from the lips of a fold; in fact, the whole structure appears like a linear blastopore, from the sides of which the mesoblast is growing out; and this as we conceive actually to be the true interpretation of the structure. Other cases occur in which the primitive groove is wholly deficient, or at the utmost represented by a shallow depression along the median axial line of a short posterior part of the primitive streak.

We may now pass to the consideration of the part of the area pellucida in front of the primitive streak.

We called attention to a change in the character of the hypoblast cells of this region as taking place at the end of the last stage. During the very early part of this stage the change in the character of these cells becomes very pronounced.

What we consider to be our earliest stage in this change we have only so far met with in the duck, and we have figured a longitudinal and median section to show it (Pl. XIII, fig. D). The hypoblast (*hy*) has become a thick layer of somewhat cubical cells several rows deep. These cells, especially in front, are characterised by their numerous yolk spherules, and give the impression that part of the area pellucida has been, so to speak, reclaimed from the *tarea opaca*. *Posteriorly, at the front end of the primitive streak,*



*the thick layer of hypoblast, instead of being continuous with the flattened hypoblast under the primitive streak, falls, in the axial line, into the mesoblast of the primitive streak* (Pl. XIII, fig. D).

In a slightly later stage, of which we have specimens both of the duck and chick, but have only figured selected sections of a chick series, still further changes have been effected in the constitution of the hypoblast (Pl. XIV, Ser. II, 1 and 2).

Near the front border of the area pellucida (1) it has the general characters of the hypoblast of the duck's blastoderm just described. Slightly further back the cells of the hypoblast have become differentiated into stellate cells several rows deep, *which can hardly be resolved in the axial line into hypoblast and mesoblast*, though one can fancy that in places, especially laterally, they are partially differentiated into two layers. The axial sheet of stellate cells is continuous laterally with cubical hypoblast cells.

As the primitive streak is approached an axial prolongation forwards of the rounded and closely-packed mesoblastic elements of the primitive streak is next met with, and at the front end of the primitive streak, where this prolongation unites with the epiblast, it also becomes continuous with the stellate cells just spoken of. In fact, close to the end of the primitive streak it becomes difficult to say which mesoblast cells are directly derived from the primitive layer of hypoblast in front of the primitive streak, and which from the forward growth of the mesoblast of the primitive streak. There is, in fact, as in the earlier stage, a fusion of the layers at this point.

Sections of a slightly older chick blastoderm are represented in Pl. XIV, Ser. I, 1, 2, 3, 4 and 5.

Nearly the whole of the hypoblast in front of the primitive streak has now undergone a differentiation into stellate cells. In the second section the products of the differentiation of this layer form a distinct mesoblast and hypoblast laterally, while in the median line they can hardly be divided into two distinct layers.

In a section slightly further back the same is true, except that we have here, in the axial line above the stellate cells, rounded elements derived from a forward prolongation of the cells of the primitive streak. In the next section figured, passing through the front end of the primitive streak, the axial cells have become continuous with the axial mesoblast of the primitive streak, while below there is an independent sheet of flattened hypoblast cells.

The general result of our observations on the part of the blastoderm in front of the primitive streak during this stage is to show that the primitive hypoblast of this region undergoes



considerable changes, including a multiplication of its cells ; and that these changes result in its becoming differentiated on each side of the middle line, with more or less distinctness, into (1) a hypoblastic sheet below, formed of a single row of flattened cells, and (2) a mesoblast plate above formed of stellate cells, while in the middle line there is a strip of stellate cells in which there is no distinct differentiation into two layers.

Since the region in which these changes take place is that in which the medullary plate becomes subsequently formed, the lateral parts of the mesoblast plate are clearly the permanent lateral plates of the trunk, from which the mesoblastic somites, &c., become subsequently formed ; *so that the main part of the mesoblast of the trunk is not directly derived from the primitive streak.*

Before leaving this stage we would call attention to the presence, in one of our blastoderms of this stage, of a deep pit at the junction of the primitive streak with the region in front of it (Pl. XIV, Ser. F, 1 and 2). Such a pit is unusual, but we think it may be regarded as an exceptionally early commencement of that most variable structure in the chick, the neurenteric canal.

The next and last stage we have to deal with is that during which the first trace of the notochord and of the medullary plate make their appearance.

In surface views this stage is marked by the appearance of a faint dark line, extending forwards, from the front end of the primitive streak, to a fold, which has in the mean time made its appearance near the front end of the area pellucida, and constitutes the head fold.

Pl. XV, Ser. K, represents a series of sections through a blastoderm of this stage, which have been selected to illustrate the mode of formation of the notochord.

In a section immediately behind the head fold the median part of the epiblast is thicker than the lateral parts, forming the first indication of a medullary plate (Ser. K, 1). Below the median line of the epiblast is a small cord of cells, not divided into two layers, but continuous laterally, both with the hypoblast and mesoblast, which are still more distinctly separated than in the previous stage.

A section or so further back (Ser. K, 2) the axial cord, which we need scarcely say is the rudiment of the notochord, is thicker, and causes a slight projection in the epiblast above. It is, as before, continuous laterally, both with the mesoblast and with the hypoblast. The medullary plate is more distinct, and a shallow but unmistakable medullary groove has made its appearance.

As we approach the front end of the primitive streak the notochord becomes (Ser. K, 3) very much more prominent,

though retaining the same relation to the germinal layers as in front.

In the section immediately behind (Ser. K, 4) the convex upper surface of the notochord has become continuous with the epiblast for a very small region. The section, in fact, traverses the front end of the primitive streak.

In the next section the attachment between the epiblast and the cells below becomes considerably wider. It will be noticed that this part of the primitive streak is placed on the floor of the wide medullary groove, and there forms a prominence known as the anterior swelling of the primitive streak.

It will further be noticed that in the two sections passing through the primitive streak, the hypoblast, instead of simply becoming continuous with the axial thickening of the cells, as in front, forms a more or less imperfect layer underneath it. This layer becomes in the sections following still more definite, and forms part of the continuous layer of hypoblast present in the region of the primitive streak.

A comparison of this stage with the previous one shows very clearly that the notochord is formed out of the median plate of cells of the earlier stage, which was not divided into mesoblast and hypoblast, together with the short column of cells which grew forwards from the primitive streak.

The notochord, from its mode of origin, is necessarily continuous behind with the axial cells of the primitive streak.

The sections immediately behind the last we have represented show a rudiment of the neurenteric canal of the same form as that first figured by Gasser, viz. a pit perforating the epiblast with a great mass of rounded cells projecting upwards through it.

The observations just recorded practically deal with two much disputed points in the ontogeny of birds, viz. the origin of the mesoblast and the origin of the notochord.

With reference to the first of these our results are briefly as follows:

The first part of the mesoblast to be formed is that which arises in connection with the primitive streak. This part is in the main formed by a proliferation from an axial strip of the epiblast along the line of the primitive streak, but in part also from a simultaneous differentiation of hypoblast cells also along the axial line of the primitive streak. The two parts of the mesoblast so formed become subsequently indistinguishable. The second part of the mesoblast to be formed is that which gives rise to the lateral plates of mesoblast of the head and trunk of the embryo. This part appears as two plates—one on each side of the middle line—which arise by direct differentiation

from the hypoblast in front of the primitive streak. They are continuous behind with the lateral wings of mesoblast which grow out from the primitive streak, and on their inner side are also at first continuous with the cells which form the notochord.

In addition to the parts of mesoblast, formed as just described, the mesoblast of the vascular area is in a large measure developed by a direct formation of cells round the nuclei of the germinal wall.

The mesoblast formed in connection with the primitive streak gives rise in part to the mesoblast of the allantois, and ventral part of the tail of the embryo (?), and in part to the vascular structures found in the area pellucida.

With reference to the formation of the mesoblast of the primitive streak, our conclusions are practically in harmony with those of Koller; except that Koller is inclined to minimise the share taken by the hypoblast in the formation of the mesoblast of the primitive streak.

Gerlach, with reference to the formation of this part of the mesoblast, adopts the now generally accepted view of Kölliker, according to which the whole of the mesoblast of the primitive streak is derived from the epiblast.

As to the derivation of the lateral plates of mesoblast of the trunk from the hypoblast of the anterior part of the primitive streak, our general result is in complete harmony with Gerlach's results, although in our accounts of the details of the process we differ in some not unimportant particulars.

As to the origin of the notochord, our main result is that this structure is formed as an actual thickening of the primitive hypoblast of the anterior part of the area pellucida. We find that it unites posteriorly with a forward growth of the axial tissue of the primitive streak, while it is laterally continuous, at first, both with the mesoblast of the lateral plates and with the hypoblast. At a later period its connection with the mesoblast is severed, while the hypoblast becomes differentiated as a continuous layer below it.

As to the hypoblastic origin of the notochord, we are again in complete accord with Gerlach; but we differ from him in admitting that the notochord is continuous posteriorly with the axial tissue of the primitive streak, and also at first continuous with the lateral plates of mesoblast.

The account we have given of the formation of the mesoblast may appear to the reader somewhat fantastic, and on that account not very credible. We believe, however, that if the view which has been elsewhere urged by one of us, that the primitive streak is the homologue of the blastopore of the lower vertebrates is accepted, the features we have described receive an adequate explanation.

The growth outwards of part of the mesoblast from the axial line of the primitive streak is a repetition of the well-known growth from the lips of the blastopore. It might have been anticipated that all the layers would fuse along the line of the primitive streak, and that the hypoblast as well as part of the mesoblast would grow out from it. There is, however, clearly a precocious formation of the hypoblast; but the formation of the mesoblast of the primitive streak, partly from the epiblast and partly from the hypoblast, is satisfactorily explained by regarding the whole structure as the blastopore. The two parts of the mesoblast subsequently become indistinguishable, and their difference in origin is, on the above view, to be regarded as simply due to a difference of position, and not as having a deeper significance.

The differentiation of the lateral plates of mesoblast of the trunk directly from the hypoblast is again a fundamental feature of vertebrate embryology, occurring in all types from *Amphioxus* upwards, the meaning of which has been fully dealt with in the 'Treatise on Comparative Embryology' by one of us. Lastly, the formation of the notochord from the hypoblast is the typical vertebrate mode of formation of this organ, while the fusion of the layers at the front end of the primitive streak is the universal fusion of the layers at the dorsal lip of the blastopore, which is so well known in the lower vertebrate types.

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*On the ORIGIN and GROWTH of the EGGS and EGG-STRINGS in NEPHELIS, with some OBSERVATIONS on the "SPIRAL ASTERS."* By ISAO IJIMA, of the University of Tokio. With Plates XVI, XVII, XVIII, and XIX.

THE following investigation was begun in the spring of 1880, and continued through the summer of the following year. Its entire course was completed in the Zoological Laboratory of the University of Tokio, under the instruction of Professor Whitman. To his advice, never-failing encouragement, and unremitting efforts to render this work as complete as time and circumstances would permit, I must ascribe much of whatever success I may have achieved.

My thanks are also due to the authorities of the University for the use of instruments and other means of carrying on my investigation, and especially to the President of the University, Mr. Kato, for permission to continue my work during the summer vacation.

For books of reference I have consulted the University Library and also that of Professor Whitman, to whom I am again deeply indebted for having put all his books at my disposal. But I wish to make it here known that I could not get access to some few works more or less connected with my subject. In the absence of such works, I hope the reader will find a sufficient excuse for any omission of due references which may have crept in. However, I have tried to make these references as complete as circumstances seem to require by the aid of citations found in those books at my command. Such references will be noted by an asterisk in the list given at the end of this paper.

My investigation has been confined to a species of *Nephelis*, probably a variety of *N. vulgaris*, Moq.-Tand. It is the only *Nephelis* found in this city, and is the most abundant representative of the *Hirudinea* found in Japan.

### *Methods of Investigation.*

1. For examination of the genital organs in a fresh condition, specimens were killed either by chloroforming or by plunging into chromic acid ( $\frac{1}{2}$  per cent.). They were pinned in a black wax trough containing water, or a salt solution ( $\frac{3}{4}$  per cent.) when swelling was to be avoided, and opened by a lateral incision, so that the dorsal body-wall could be removed. For certain purposes, it is advantageous to allow

the tissues to swell in water; but for accurate measurements or sections, leeches were opened in salt solution. The fresh ovaries were carefully taken out with a pair of forceps, put in dilute glycerine or osmic acid ( $\frac{1}{6}$  per cent.), and dissected with needles under a dissecting microscope.

2. For sections of an entire leech, it was found best to make transverse cuts at convenient places, and to harden in chromic acid ( $\frac{1}{3}$  per cent.) three or four hours. In order to remove as much of the acid as possible, the parts were next placed in water for several hours, the water being renewed two or three times, and then the hardening completed in alcohol, using first weak, then strong, and finally absolute alcohol. Dilute Beale's carmine was used for staining (twenty-four to thirty hours). After colouring, they are again to be passed through weak, strong, and absolute alcohol.

3. For hardening ovaries and egg-strings the above method was sometimes used; but the object was exposed for a shorter time (one hour) to the action of a weaker acid solution (ca.  $\frac{1}{5}$  per cent.).

A more convenient method, with about equally good results, was to harden in Kleinenberg's picro-sulphuric acid (two to three hours), succeeded by 70 per cent. (two to three hours) and 90 per cent. (two to three hours) alcohol. Coloured in Ranvier's picro-carmine, washed, and again hardened in alcohol as before.

4. For section-cutting, specimens taken from absolute alcohol were thoroughly soaked in clove oil or bergamot oil. Embedded in paraffine, to which a little pig's lard had been added. Cut by means of a microtome. Paraffine removed from sections by benzine, and clarified with creosote or clove oil. Or, paraffine removed and sections clarified with a mixture of four parts of essence of turpentine and one part of creosote. Mounted in Canada balsam.

5. For surface views of the ovary-wall, the ovary was first split open along one side with needles, then stretched flat on a slide, and hardened and coloured as in the case of ovaries and egg-strings.

I have also obtained very good preparations by hardening in  $\frac{1}{6}$  per cent. osmic acid (ten minutes), coloured with picro-carmine, and mounted in glycerine.

6. For the examination of early changes in mature eggs, I have adopted the method used by O. Hertwig ('Morph. Jahrb.,' B. iii, p. 9) with very slight modifications. It is as follows:—Treated with acetic acid (1 or  $1\frac{1}{2}$  per cent.) for about ten minutes, the acetic acid replaced by absolute

alcohol (ca. one hour), and examined in a mixture of equal parts of glycerine and potassic acetate, or in dilute glycerine.

Such preparations may sometimes in a few days become dark, but they can be again clarified by a fresh supply of the mixture or the dilute glycerine. Eggs thus hardened can be examined in any desired position by carefully rolling them over under the cover glass, beneath which there must be a hair or a piece of paper to avoid hard pressure.

Specimens can be coloured with Beale's carmine after hardening in absolute alcohol, but these show no better than uncoloured ones.

### I. *Reproductive Organs.*

I must here express my deep regret that I have not been able to consult a memoir of M. Robin (No. 1), in which he has described the sexual organs of *Nephelis*. I have also been unable to obtain two memoirs of an earlier date, by F. Müller (No. 2) and Leydig (No. 3).

The form and structure of the male organs have been for a long time tolerably well known; the same cannot be said, I believe, of the female organs. I shall therefore first define briefly the position of the genital orifices, and then pass to a more detailed account of the sexual organs, limiting myself in the case of the male organ to such remarks as the context seems to require.

The male organ opens externally in front of the female organ, in the median ventral line, as is always the case in all hermaphroditic *Hirudinea*. The male orifice (*m. o.*, fig. 1, Pl. XVI) is situated in the 36th ring, and the female orifice (*f. o.*, fig. 1) between the 38th and the 39th ring, the entire leech being composed of 106 rings. The former is comparatively large and prominent, and its edge is thrown into radial rugæ. The latter is hardly perceptible, except in hardened specimens, being a simple minute pore in the interannular line.

*The Male Organ.*—The testicular sacs (*t.*, fig. 1), beginning in the plane of the 71st—73rd ring, about opposite the junction of the gastric portion with the yellow intestinal division of the alimentary canal, extend backward to near the end of the body. Their form and arrangement agrees perfectly with the European *Nephelis*. They are spherical bodies arranged compactly together, two to three broad, in two elongated masses, disposed on each side of the nerve-chain. They vary somewhat in size, but not exceeding 1 mm. in diameter during the winter period of inactivity.



Their number is not limited to one pair in each segment, as in most leeches. I once counted as many as sixty-two on one side of the median line: It is impossible to say precisely how many occur in each segment; but as they extend through about six segments, the average number cannot be far from twenty on one side of the median line.

From what I could learn from my sections and dissections, I am inclined to think that the testicular sacs are sessile and not connected to the vasa deferentia by special stalk-like ducts, as in *Hirudo* and some other leeches.

The seminal ducts, or vasa deferentia, in this part of the body are minute tubes, which pass along the under side of the testicular sacs (*vd.*<sub>1</sub>, fig. 1). Just in front of the foremost testis each seminal duct abruptly enlarges to a diameter of about .7 mm., and at the same time assumes the form of an elongated coil, which reaches forward to the hind end of the ovaries. It is in this portion (*vd.*<sub>2</sub>, fig. 1) that one always finds, especially in the latter part of winter, before copulation has taken place, numerous bundles of spermatozoa; and it is this portion which can be seen through the body-wall of the living specimen, as a white convoluted streak.

From near the posterior end of the coiled portion the diameter of the seminal duct gradually diminishes as it passes forward, reaching its minimum near *vd.*<sub>1</sub> (.12 mm). At the level of the fifth ganglion (counting the one immediately behind the subœsophageal ganglion as the first) the seminal duct makes a bend and passes back into one of the horns of the ejaculatory organ ("la bourse," Moquin-Tandon; "zwei-hörniger kurzer Sack," Leuckart).

It will be necessary to give here a brief description of this organ, in order to render intelligible what I shall have to say concerning copulation. It consists of a main median portion and two lateral horns, the form and position of which may be seen from fig. 1. The lumen of the median portion passes directly into that of each horn, so that the cavity of the organ corresponds nearly with its external form. The wall may be briefly described as consisting of an outer thick muscular layer and an inner lining epithelium of a glandular character.

The ejaculatory organ is supplied with a mass of unicellular glands, arranged belt-like around it, just below the junction of the horns. This glandular mass is analogous to that which is found in other leeches occupying a similar position, and, according to Leuckart (No. 4, p. 675, vol. i), it may be regarded as a sort of Prostata, whose granular



secretion unites the spermatozoa into spermatophores. The common cavity in the main portion of the ejaculatory organ opens below into a short flask-shaped canal, the neck of which terminates in the male orifice, as seen in my sections.

I have not made a thorough study of the development of the spermatozoa, but, from what I have seen, it corresponds very closely with what Bloomfield (No. 5, p. 83) has observed in *Lumbricus*.

*The female organ.*—All of the essential anatomical features of this organ are well known, but the same cannot be said of its finer histological structure. The chief object in this portion of my study has been to determine the place and manner of origin of the egg strings, and in doing this I have found it necessary to make a thorough study of the wall of the ovaries at different seasons.

Before giving the results of my histological observations a brief anatomical description will be in place.

The external orifice (*f. o.*, fig. 1, Pl. XVI) leads into a short vaginal duct (ca. 4 mm. in length), which takes a vertical direction, and passes, at its upper end, into the two ovarian sacs (*ov.*). These sacs are in free communication with each other at their point of junction with the vagina, so that the contents of one may be easily forced into the other.

The ovarian tubes take at first opposite directions, nearly at right angles to the longitudinal axis of the body, but, soon bending backward, pursue nearly parallel courses through about two segments, beneath the edges of the lower œsophageal fold, and just above the vasa deferentia. Then gradually approaching each other they take a median position, and for the remainder of their backward course lie in close contact. They extend back to the 55th—63rd ring (one, either right or left, always extending farther than the other), where each, having attained a maximum diameter, makes a close bend upon itself, thus forming a pair of loops.

From the posterior end of each loop the ovarian tube retraces its course, following closely the path already marked out. Several twists are to be seen in the posterior two thirds of each loop, but in the anterior third the course is more direct. At about the level of the vaginal orifice the terminal portion of each tube assumes a dorsal position, above the œsophagus, where, dwindling to a mere point, it is lost in other tissues. The dorsal termination (*t. ov.*) may easily be seen after peeling off the dorsal wall of the body. According to Moquin-Tandon (No. 6, pl. iv) these terminal

portions meet and coalesce in Trocheta ; but I have satisfied myself by dissections, as well as by microscopical observations, that no such union exists in our *Nephelis*. In this respect what I have seen agrees entirely with Professor Leuckart's (No. 4, p. 677-678) statement, that these tubes have no communication with each other, except at the point of their common outlet. Moquin-Tandon has represented the ovaries of *Nephelis* as a single tube in the form of a collapsed ring.

*Contents of the Ovaries.*—Within the ovaries there is a number of so-called egg-strings, some of which are free, while others (younger) are not yet detached from their place of origin. Of these I will have to speak in detail further on. The free egg-strings float in a clear fluid, probably of a protoplasmic nature. The origin of the fluid is unknown, but it is certain that it is fed, partially at least, by the dissolution of some of the bodies which are found floating in it, and which are presently to be described.

Whatever other purposes this fluid may subserve, there can be no doubt that it holds nutritive substances in solution, which are appropriated by the growing egg-strings.

Throughout the winter months I found ripe eggs floating in the ovaries, but am unable to say what becomes of such eggs. Possibly they may remain in a comparatively quiescent state until the time for laying approaches. Of one thing there is no doubt, viz. that ripe eggs, after the formation of the archiamphiaser (Whitman), are retained for a considerable length of time in the ovaries, during which time no discernible changes take place. A similar fact has been noted in *Clepsine* by Professor Whitman (No. 7, p. 231), who found that ripe eggs were often retained four or five days within the ovaries.

Leydig (No. 3, p. 125) found in the ovary of *Piscicola* many eggs that were undergoing regressive changes, and also some empty egg-membranes. O. Hertwig (No. 8, pp. 14 and 19) also mentions the occurrence of such eggs in *Hæmopis*. In the case of *Nephelis* he states that some of the ripe eggs break up and serve evidently as nourishment for the rest. My observations accord fully with these statements. I have often seen many eggs with their lecithin granules either in irregular clumps or in otherwise disordered conditions. I have also found empty membranes of broken eggs.

In the ovarian fluid there is also a large number of free cells, varying in size, but averaging ca. .015 mm. in diameter. These are found floating about, or else packed among spermatophores (?). They contain comparatively large, highly

refractive granules, on which account their nuclei are not to be easily seen in a fresh condition. Exposed to fumes of osmic acid and coloured with picro-carminine their nuclei can be clearly made out. Nuclei thus demonstrated gave an average diameter of  $\cdot 005$  mm. No nucleolus could be discovered in the nucleus, nor could any cell-membrane be recognised. What significance these free cells possess must be left undecided for the present, but with regard to their origin I may make a suggestion. Pressing either an egg-string or the germogen under the cover-glass, a crowd of nuclei and also cells escape together with granular protoplasm. Some at least of the cells thus set free have exactly the appearance of those found in the ovarian fluid. This fact seems to favour the supposition that these free cells are originally egg-cells, which have become free by the breaking up of egg-strings or of the germogen. Leydig has remarked that these cells probably arise from the epithelium of the ovary-wall. If the above supposition be true his remark is correct in so far that the free cells can be indirectly traced to the epithelium. Notwithstanding my special search, I have not met with any case in which these free cells could be traced directly to the epithelial lining of the ovary.

Together with free cells are sometimes found apparently free nuclei, which stain deeply in picro-carminine. These perhaps have the same origin as the free cells.

Highly refractive, yellowish granules of the same appearance as yolk-granules, are also abundant in the ovarian fluid.

Several massive aggregations of spermatozoa (spermato-phores?), each containing an enormous number of spermatozoa, are always found in each ovary about the time the laying season begins. It seems probable that the number greatly exceeds that of the spermatozoa which actually penetrate the eggs, and that the superfluous ones dissolve sooner or later, and thus contribute something towards the formation of the ovarian fluid.

## II. *Copulation, Abnormal Copulation, &c.*

*Copulation.*—Although it still remains doubtful whether copulation ever occurs in *Clepsine*, this act has been observed in many other members of the leech-family, and described by various authors.

In the case of the Medicinal Leech, some authors (No. 6



pp. 167-168) have stated that the head of one individual is turned towards the posterior end of the other, in such a manner as to render possible reciprocal fecundation. Johnson, according to Moquin-Tandon (No. 6, p. 168), asserts that the process is the same in *Nephelis* as in *Hirudo*.

According to Ébrard (No. 9, p. 102), the two individuals (*H. medicinalis*) always have their heads turned in the same direction, so that, if both individuals are fecundated, they must be so successively, and not simultaneously. Other observations of Ébrard make it probable that only one individual is fecundated during a single copulation. This accords with my observations on *Nephelis* which are as follows:

The anterior portions of two individuals, attached by their suckers to the glass-vessel near each other, are spirally entwined in such a manner that the ventral surfaces of their genital bands are always brought into apposition. They maintain this position for a considerable time, now and then changing the direction of their winding, and relaxing or tightening their hold. Sometimes the act is of short duration, and two or three times renewed at short intervals. At other times, and when disturbed, the act ceases altogether, or else they combine with other individuals. It is evident that there can be no reciprocal fecundation while the leeches are coupling in the position above described.

As there is no intromittent organ it is probable that the male-orifice with its prominent muscular lips clasps the female orifice, while the spermatozoa are forced onward by the action of the ejaculatory organ.

I am unable to say precisely at what time of the year copulation begins; but I found spermatozoa in the ovaries of one leech on the 20th of February, for the first time in this year. The act of coupling, so far as my experience goes, takes place almost always in the morning.

*Abnormal Copulation.*—I have often found individuals with a small, two-horned, whitish object adhering to some portion of the genital band. The position of this cornuous body was always on or near the genital band, sometimes on the dorsal surface, sometimes on the extreme margin, but more frequently on the ventral surface than elsewhere. It consisted of two thin-walled bottle-shaped tubes (ca. .5 mm. long), the broader ends of which were inserted, close to each other, into a small disc-like portion. This portion, the margin of which presented a villiform appearance, was partially embedded in the epidermis. Around the disc was a discoloured area, which proved to be, on examination of



sections, a macerated portion of the epidermis. The two bottle-shaped tubes were filled with spermatozoa, and opened by means of two distinct holes in the disc. From each of these openings a stream of spermatozoa was found, penetrating to a considerable depth into the underlying tissues. In section the substance of the two-horned body appeared dotted, and longitudinally striated, but I was unable to recognise any cellular structure.

For a long time I was much puzzled as to the meaning of all this, but from further observation was led to regard it as a case of abnormal or unsuccessful copulation.

When disturbed during the act of copulation, the two leeches usually separate immediately; but in one instance they did not separate even after putting them into chromic acid. On examination I found that the female orifice of each leech was not in contact with the male orifice of the other, but that each individual was attached to the ventral surface of the other by its male orifice, the female orifice remaining free. On separating them by force, each male orifice left the two-horned object on the body of the other.

In another instance that came under my notice, only one individual had already deposited the two-horned body, while the other had a mass of spermatozoa hanging from its male orifice. The latter was dissected, and the two-horned body found occupying the whole interior of the ejaculatory organ, with the cavity of which it exactly corresponded in shape. It came out without any resistance. It thus became evident that the two-horned body belongs to the interior of the ejaculatory organ. That it forms no permanent part of the male organ, seems evident from the fact that sections made in the winter show no trace of such a body. I have not thus far been able to determine whether this body forms in the case of normal copulation also. As to its mode of formation. I have nothing to offer except the conjecture that it may be the hardened secretion of some of the glands of the ejaculatory organ.

On many leeches were found scars, which very likely may have been the marks left by these peculiar bodies.

It is hardly to be doubted that the normal mode of charging the ovaries with spermatophores is through the female orifice. It would certainly be impossible for the spermatozoa to find their way into the ovaries in many of those cases which we have described as abnormal, especially where the injection takes place far in front of the male orifice.

*Cocoons, and their contents.*—If well fed with fish-meat

or larvæ of mosquitoes, *Nephelis* will lay eggs in abundance. Leaves of *Nuphar Japonicum* were kept in the vessels, and on these the cocoons were always placed. In order to save fresh-laid cocoons from being eaten up, it was necessary to isolate the cocoon-laying individual, by removing it together with the leaf to a separate vessel. This method was found more convenient than keeping each in a different vessel.

As to the time of deposit, I obtained the first cocoon of this year on the 12th of April; and last year I found freshly laid cocoons as late as September 29th.

According to A. Schneider (No. 10, p. 256), *Nephelis* reaches sexual maturity in the spring of its second year, and dies in the autumn. I have made no decisive observations on this point, but what I have seen has led me to think that *Nephelis* is not a biennial animal.

Within the cocoon is contained a fluid (Eiweiss of German authors), which in a few hours after deposit hardens to a transparent jelly-like consistency. If the contents of a freshly-laid cocoon be examined, there will be found floating in it, besides eggs, a considerable quantity of spermatozoa, in a more or less broken condition, and also some pale looking corpuscles, varying in size from a mere granule to a body of .017 mm. in diameter. These bodies disappear when the fluid is exposed directly to water or acetic acid. Caustic potash dissolves them, and therefore, as was pointed out by Rathke (No. 12), they cannot be oil globules. From the fact that later they increase both in bulk and number, it is evident that they are formations of the fluid itself.

Rathke mentions the presence of short, ill-defined fibres which run in no definite direction. I have found nothing of this nature, and as he makes no mention of spermatozoa being present, I think he may have mistaken the fragments of spermatozoa for fibres.

Sometimes free cells, that were found floating in the ovarian fluid, will also be seen in the fluid.

All the bodies above mentioned persist up to the time when embryos are considerably developed, but probably dissolve finally.

### III. *The Structure of the Ovary-wall.*

In order to learn where and how the egg-strings arise, I have found it necessary to make a careful study of the minute structure of the ovary-wall, and this study has, besides determining the point in question, brought out interesting histological peculiarities.

The ovaries are for the most part embedded in a soft connective tissue, in which many nucleolated nuclei ( $\cdot 0025$  mm. in diameter) are to be seen (*n.*, fig. 4, Pl. XVII), but no distinct cell outlines.

The ovary-wall separated from this tissue has a thickness of about  $\cdot 02$  mm., and is composed of two distinct layers—an internal layer of cells (*i. ep.*, fig. 4) arranged loosely and one cell deep, and an external layer (*ex.*, fig. 4) composed of muscles and cells.

In section the external layer is seen to be by far the thicker portion; and it may, for sake of convenience, be described as composed of four strata; although these, with the exception of the muscular strata, are not distinct. These four strata, beginning with the outermost, are (1) the circular muscle-fibres (*cir.*, figs. 3 and 4); (2) a stratum composed of loosely-packed cells and a network of vaso-fibrous tissue (Lankester) (*v. f.*, fig. 3); (3) the semi-circular muscle-fibres (*s. cir.*, fig. 3); and (4) a cellular stratum, which differs from the second stratum only by the more or less complete absence of vaso-fibrous tissue. The cells of No. 2 and No. 4 really constitute one layer, being separated only by the semi-circular muscle-fibres.

The external stratum of circular muscle-fibres is seen in surface in fig. 3, in longitudinal section in fig. 4, and in transverse section in fig. 11. These fibres, varying in width, but having a maximum of about  $\cdot 02$  mm., have a transverse direction, completely encircling the ovarian tube. They take nearly parallel courses, except where they bifurcate or anastomose, and never overlap one another. Thus their arrangement forms a sort of network, the meshes of which are transversely elongated. In transverse section they present elongated elliptical figures, showing that they are flat rather than round. Histologically speaking, they belong to the unstriated class of muscle-fibres. The sarcolemma is comparatively thick, and presents a homogeneous appearance. The central portion consists of finely granular protoplasm, which encloses in widened places and at comparatively long intervals, large elliptical nuclei ( $\cdot 011 \times \cdot 007$  mm.), in each of which a nucleolus is generally to be seen.

In the superficial portion of the second stratum is found a system of pigmented fibres, having a reticular arrangement, as is shown in fig. 3, *v. f.* Although by far the greater part of these fibres lie just beneath the circular muscle-fibres, here and there branches are seen to penetrate deeper into the wall. The larger fibres are evidently hollow, the wall being formed of pigmented cells; but others appear to



be solid. Professor Lankester (No. 14, p. 313) regards the tubular and the solid fibres, together with the hæmatophorous vessels, as three permanent varieties of the vaso-fibrous tissue. He found a direct continuity between the first two kinds of fibres and the third, which corresponds to what I have seen.

The black pigment granules (brown in *Hirudo medicinalis*), if the action of the acid has been weak, remain uncoloured by carmine; but in cases of strong action, or when hardened in Kleinenberg's picro-sulphuric acid, there is no trace of colour left, and the nuclei become very distinct. These nuclei (ca.  $\cdot 004$  mm. in diameter) were round or oval in outline, and showed no nucleoli. The widest portion of these fibres, which is always near the junction of some two or three strands, measures about  $\cdot 015$  mm.

I am unable to offer any suggestion in regard to the significance of these fibres. In the case of the medicinal leech, Professor Lankester is inclined to think "that there is actually a continual development of off-shoots of the brown pigmented system of fibres into thin-walled hæmatophorous vessels, the nuclei entering the blood stream when communication is once established, and the brown granular wall becoming transparent and structureless by the absorption of its granules." Whether this view is applicable to the ovarian system of *Nephelis* I am quite unable to say. I have thus far been unable to recognise any blood-vessels in the ovary wall.

It will aid in the further description of the ovary to introduce here some explanatory remarks and definitions. As the ovary-wall does not present complete uniformity, either in thickness or in composition, in its entire circumference, it will be necessary to distinguish between different sides by the use of different terms. That side of the ovarian tube which, at the fore end of the tube, always looks outward towards the margin of the body, but which elsewhere may face in any direction, may be called the *rachal side* (*ra. s.*, fig. 2), since it is always thickened along its median line. The longitudinally-thickened portion of the wall will be designated as the *rachis* (*ra.*, fig. 4, Pl. XVII). The opposite side of the ovarian tube will be called the *mediad side* (*m. s.*, fig. 2), although it cannot of course always lie nearest the median line of the body. In fig. 3, Pl. XVI, the middle of the rachal side is marked A, and the corresponding mediad line by B. Although the position of these lines varies in different parts of the ovaries, owing to the winding of the tubes, they always preserve the same relation to each other.



The rachal side bears what I have already called the germogen (*ger.*, figs. 2 and 4), and is thus easily recognised.

We may now conclude our description of the second stratum. In addition to the pigmented system of fibres before described and the loosely packed cells, there occurs on the rachal side a system of longitudinal muscle-fibres. The few fibres (1—3 in number) representing this system run nearly parallel along the inner limit of the second stratum, thus forming a sort of muscular axis to the rachal thickening (*long.*, figs. 3 and 4). Like all the muscle-fibres of the ovary-wall, they are broadest at the points occupied by the nuclei. They are of considerable length, and taper toward either extremity. Whether they end in a point, or ultimately anastomose with their fellows, as in the case of the circular muscle-fibres, I am not prepared to speak with certainty.

The third system of muscles, constituting the third stratum, presents some interesting peculiarities. The transverse fibres are disposed in two longitudinal series, each of which encircles one half of the ovarian tube, beginning in one—say the rachal line—and terminating in the other (mediad line). The nuclei of each series of semicircular fibres (fig. 3, *s. cir.*) fall nearly in one line, situated about midway between the two extremities. The line of nuclei lies somewhat nearer to the mediad (B) than the rachal line (A), which is explained by the fact that the mediad end of each fibre is more strongly curved than the opposite end. These fibres taper from the broad middle portions (ca. .015 mm.) which bear the nuclei towards both ends. The course and arrangement of these will perhaps be best understood if we confine our attention for a moment to a single strand. According to what has been said, the position of the rachal and the mediad line in the anterior portion of each ovary is such that a horizontal plane joining these two lines would divide the tube into an upper and a lower half. If now we take a fibre in the upper half and, starting from the highest point, which coincides nearly with the nucleus, follow it towards the mediad end, we find not only that it descends as it describes the arc of its quadrant, but also that it gradually bends forward until, in the neighbourhood of the mediad line, it has a longitudinal direction. A curve of double curvature is thus formed. The mediad portion usually bifurcates one or more times, and the branches either anastomose with branches belonging to the opposite series, or, after crossing them, terminate in points. On the other side of the nucleus a similar curve is formed, the only difference

being that the rachal end is bent forward less strongly than the mediad end.

The course of these fibres gradually changes towards the termination of the ovary (fig. 1, *t. ov.*), in such a manner that they finally assume a longitudinal direction, the ovary itself dwindling to a point. The entire fibre, as above described, is composed of two somewhat unsymmetrical halves, which form together a complex curve, or a curve which faces forward and inward at the same time.

This system of muscle-fibres, as before stated, lies between the second and the fourth stratum.

The outlines of the cells which make up the fourth and most of the second stratum are not represented in fig. 3, but are more or less distinct in fig. 4.

They do not have the angular form of closely-packed cells, but are more or less rounded, and often separated by considerable intervals. As is seen in fig. 4, the cells of the second and the fourth stratum make up the greater part of the ovary-wall. In most places the cells are two deep; but here and there a single cell appears to represent all that can be seen of the two strata. The four strata just described constitute what may be called the *tunica propria* of the ovarian tubes.

In regard to the fifth stratum, or the lining epithelium (*l. ep.*, fig. 4), I have found that it agrees perfectly with what Leydig (No. 3, p. 549, mentioned in No. 15, p. 63) has described in the case of *Piscicola*. This stratum is composed of cells which we may regard as epithelial, although they do not present the form or arrangement characteristic of most epithelial layers. Instead of forming a continuous sheet of closely packed cells, they are so loosely arranged that wide spaces are left between them, except in the longitudinal area (*ger.*) borne by the rachis, where they give rise to egg-strings. Outside this germinal area the cells have an elongated spindle shape, the widest part of which is filled by a nucleus  $\cdot 008$  mm. in diameter. Sometimes, especially in or near the germinal area, which I shall call the *germogen*, cells with two nuclei are found. These cells are usually so arranged that the longer axis has a longitudinal direction. In section they appear as small spherules attached to the wall of the ovary (*l. ep.*, fig. 4), with intercellular spaces measuring about  $\cdot 03$  mm. in width.

As to the part played by the different muscle-fibres before mentioned, it seems probable that the circular and semi-circular fibres may be capable of producing peristaltic con-

tractions, which travel from behind forward. The contraction of the semi-circular fibres, whose extremities are prolonged forward, would tend to push the contents of the ovaries in the same direction. The ovarian tube could be shortened on the mediad side by means of the longitudinally prolonged ends of the semicircular fibres; while on the rachal side, where these fibres are very little prolonged, the shortening would be mainly effected by the contraction of the longitudinal fibres.

#### IV. *The Germogen.*

If a fresh ovary be examined, there will be seen, besides free egg-strings, a longitudinal ridge of cells, some parts of which are beginning to take the form of egg-strings. The entire ridge, which is a massive thickening of the lining epithelium all along the inner surface of the rachis, may be called the *germogen*.<sup>1</sup> This is shown half-diagrammatically in fig. 2, in section in figs. 4 and 11, and as partially seen through the ovarian tunic in fig. 3, *ger*.

It is not strictly speaking a single ridge. It may be said rather to be composed of a main median ridge, with smaller ones on each side (fig. 4). But these ridges, or strings, are very irregular, often diverging and uniting in such a manner as to leave thin or open mesh-like spaces (fig. 3). Sometimes one of the smaller strings is continued into the larger, or *vice versâ*. The main string is irregularly cut here and there into parts destined to become egg-strings (fig. 2). The parts have no well-defined form or limits at first, but assume gradually the form of egg-strings as they gain in size.

When one of these, after attaining its full size, becomes detached, the smaller strings may continue to grow, unite in one string, and thus replace the detached portion. The germogen is more massively developed where the calibre of the tube is greatest.

The germogen is simply a massive enlargement of the lining epithelium, and, so far as I can learn, no other cell-elements take any direct part in its formation. That the loose fusiform epithelial cells actually give rise to germ-cells there is no doubt. Transitional forms are met with abundantly both in sections and surface views (fig. 3). But as there is no membranous wall between the epithelial stratum

<sup>1</sup> I borrow this term from Balfour ('A Treatise on Comparative Embryology,' vol. i, p. 14), who uses it, in a restricted sense, to designate a nucleated mass of protoplasm (syncytium) in which cell-outlines are apparently absent, but which give rise to ova. I have taken the liberty to apply the word to a case in which cell-limits are more or less distinct.



and the inner cellular stratum of the ovarian tunic, I cannot deny that there is a possibility that the cells of the latter enter the germinal mass; but I can say that I have never been able to find any evidence whatever in favour of such a view. In sections of the rachal side of the ovary-wall there is always to be seen a distinct boundary line between the germogen and the inner stratum of the tunic; and this is true whatever be the stage of development of the germogen. Fig. 4 shows this line under even the youngest, least thickened portions of the germogen. In this figure the central cell-string is already beginning to get loosened from the wall, as is shown by the open nature of the attached side. I am inclined to think that when once the massive germogen has been formed, the enlargement of the strings is mainly due to the growth and multiplication of its own cells, and that the fusiform epithelial cells only take part in forming the very youngest portion of the germogen.

In regard to whether the germogen consists of cells, or represents merely a nucleated mass of protoplasm (syncytium), it must be confessed that the appearances are not all on one side. In the fully formed free egg-string the cell-limits towards the ends are quite distinct, as is shown on the left side of fig. 11, Pl. XVIII. In the central portions, where egg-formation is going on, the outlines are sometimes distinct, and sometimes apparently wholly obliterated. But as this obliteration is limited to the egg-producing portions, we may regard it as an evidence that some of the cells are in process of absorption. Such cells would appear to lose distinct outlines and to coalesce, thus forming a protoplasmic matrix, in which are seen, besides the nuclei of the original cells, germ-cells and ova in different stages of development. The protoplasmic portion thus formed—seen abundantly in figs. 12, 13, and 14—serves undoubtedly as food for the proper germ-cells and the definite ova. If we look at the younger portions of the germogen, seen in fig. 4, we find the cell outlines very ill-defined; but this may be considered the fault of the preparation, since in other cases, as in fig. 11, such outlines are quite distinct. In this figure, as in fig. 4, portions of the strings are seen to be very loosely attached to the ovary-wall by a meshwork of protoplasm, the meshes growing larger as the strings become heavier. This protoplasm may also be regarded as broken-down cell-substance. The germ-cells, of which the germogen is largely made up, possess large oval nuclei, usually very distinct in outline, in which one to several nucleoli are generally seen. In the central portions of the egg-strings, even



before they become detached from the ovary-wall, a series of changes begins in some of the germ-cells which ends in the formation of the primordial ova.

The first step consists in the multiplication of the nucleus with a simultaneous enlargement of the cell-substance. Several cases are seen (in fig. 4) of cells containing two nuclei. Fig. 5 represents a string which is about ready to become free. Here one cell with three nuclei is seen on the right.

A glance at fig. 4 will make it plain that the single nuclei vary considerably in size ( $\cdot 006$ — $\cdot 009$  mm.). The size of twin nuclei fig. (4) also differs much ( $\cdot 007$ — $\cdot 013$  mm.). The nuclei not only increase in size but also present an aspect quite different from that of most single nuclei. They are less regular in form, clearer, and less strongly stained with carmine. The nucleolus is either absent or broken into small pieces. The twin or multiple nuclei lie embedded in an area of granular protoplasm, which differs in no particular from the smaller areas containing each one nucleus. While the growth of the germ-cells into multi-nucleated masses is provided for, at least in part, by the breaking down of other cells, I do not think that the pabulum thus provided is taken in massive forms, or that whole cells are swallowed after the manner of an *Amœba*. There is always a distinct boundary to the multi-nucleated masses, although no proper membrane. There is abundant evidence of the division of nuclei, but none at all that these clusters of nuclei arise by the coalescence of as many cells. In fig. 5 there is one much elongated mass of protoplasm with three nuclei. It is not impossible that this mass represents two enlarged germ-cells in process of coalescing, but it is more probable that it arose from a single germ-cell.

The further history of this mulberry stage of the nucleus must be learned from sections of the free egg-string.

#### V. *The Egg-strings and the Formation of the Primordial Ova.*

Each ovarian tube always contains a number (3—5) of free egg-strings (fig. 2), the different parts of which show different stages in the formation of ova.

The egg-string is a club-shaped body, from 2.5 to 4.8 m.m. in length, and from  $\cdot 35$  to  $\cdot 50$  mm. broad at the widest part. As will be seen from fig. 6, the wider portion is much nearer one extremity than the other, and it is in this portion that the more advanced stages of the ova are found. The larger

number of strings are usually found in the wider portion of the ovary, near the hind end of the loop. Sometimes the broader, sometimes the narrower end is turned forward, and occasionally one is found doubled up.

Sections passing through the broader end of the string (fig. 10) show us the same stages that we have already noted in the unliberated string, together with others differing from them in having larger clusters of nuclei (*y.*, *fol.*).

Fig. 12 represents a section somewhat nearer the broader part of the string, and here we find what we may call a primordial ovum. Around one of the nuclei in the larger multi-nucleated mass a distinct cell-like outline has appeared, enclosing a mass of protoplasm much clearer than the surrounding protoplasm. Clear areas are seen also around some of the other nuclei, but no distinct boundary line.

Still nearer the widest part of the string are found more advanced stages. In fig. 13 are represented all the stages thus far described, and three young follicles, as we will now call those sac-like areas enclosing nests of nuclei with ova. As the ovum grows at the expense of the protoplasm enclosed in the follicle, it soon comes to occupy nearly the whole follicle, the remaining nuclei being crowded into the periphery (fig. 14). As a rule, follicles with large ova are situated near the surface of the string, while very young follicles and crypts, containing only nests of indifferent nuclei, are found everywhere between the centre and the periphery of the string.

From the widest portion of the string towards either extremity, younger and younger follicles are met with, passing finally into simple germ-cells near the ends. There is little difference between the two extreme portions of a string, except that the formation of ova is rather more energetic in the broad terminal portion than in the other. Toward the smaller end of the string the outlines of the germ-cells become somewhat sharper than elsewhere (as shown on the left of fig. 11), and diminish in size (compare figs. 7 and 8, taken through the planes A—A and B—B in fig. 6). It may also be noted that in fig. 7 each nucleus has a single nucleolus, while in fig. 8, and from here onward, the nucleolus appears in several pieces.

The egg-string appears not to be invested with any distinct membrane at any period of its history. In sections the hardened periphery differs in no particular from the sub-jacent portions.

In the laying season the larger egg-strings are considerably longer than any found in winter (measuring from 6 to

7 mm.), and have lost the club-shape seen in fig. 6. The follicles towards either extremity have become larger, and the string has in consequence assumed a nearly uniform width for the greater part of its length. It is during this season that we meet with strings which are in process of dissolving, and among the loosened elements of such strings are always found spermatozoa. It has occurred to me that possibly such appearances may have led Robin to the theory of "ovo-spermatophores."

A tolerably distinct membrane appears to envelope the ovum very soon after the cell-outline is seen. I have represented this membrane with double outline in fig. 13, but I am disposed to admit that I have somewhat exaggerated this point. The drawing conveys, however, a sufficiently accurate notion of the appearance of the membrane. The membrane evidently arises from the protoplasm; but whether from the enclosed cell-body or from the enveloping protoplasm of the follicle, I am not able to say.

With the growth of the ovum the germinal vesicle increases in size, but not in the same proportion as the ovum. From the following measurements it will be seen that, while the diameter of the egg has increased about elevenfold, that of the germinal vesicle has only quadrupled.

| Ovum.    | Germ. vesicle. |
|----------|----------------|
| ·015 mm. | ·01 mm.        |
| ·062 "   | ·02 "          |
| ·095 "   | ·03 "          |
| ·17 "    | ·04 "          |

*The Yolk Nucleus.*—In eggs that have attained the size of about 1 mm. I have frequently found one or more corpuscles embedded in the vitellus, usually near the periphery. In a fresh-laid egg I once counted as many as fifteen of these bodies. They vary considerably from a spherical form, are quite refractive, and present a granular appearance. They differ also much in size, the largest observed measuring ·016 mm. Their variable number and total absence in some eggs show that they have no special morphological value.

I am inclined to think that they are analogous to the yolk nucleus ("Dotterkern") found in the Amphibian egg. O. Hertwig (No. 8, p. 37) is of the opinion that this body is simply a peculiar aggregation of food stuff, and proposes to call it "Dotterconcrement."

It will be seen from the foregoing that the origin of the egg in *Nephelis* differs somewhat from the same in *Piscicola*, as described by Ludwig (No. 15, p. 63-67). It may be well here to recapitulate briefly my observations on this subject,

both for the sake of giving a connected account of the same, and for comparison with Ludwig's statements.

A single germ-cell without a proper membrane, but tolerably clearly outlined, enlarges, and its nucleus multiplies by division, thus producing a large multi-nucleated mass or cell. Around one of these multiple nuclei a well-defined area of clear protoplasm, separated from the remaining protoplasm by an inchoate membrane, soon appears. The primordial ovum thus formed assumes a spherical shape, and the membrane gradually becomes double-contoured. The ovum grows at the expense of the contents of the follicle, crowding the free nuclei against the follicular wall as it grows in size.

The contents of the follicle enlarges at the expense of the adjacent cells, which coalesce to form a protoplasmic mass, with free nuclei around the follicle. Thus, the follicle with its protoplasmic contents, the free nuclei and the ovum, all arise from a single germ-cell.

The follicle has no definite wall in the form of a layer of cells, as in the case of higher animals, but represents simply the area formed by the expansion of a single cell—an area bounded by a hardened area of the elements of the egg-string.

The formation of the ovum of *Nephelis* is in one particular an abbreviated form of what occurs in *Piscicola*. In both cases the whole series of events transpires within the limits of a single cell. In *Piscicola* free nuclei are also formed, but eventually these nuclei become the centres of so many cells, while in *Nephelis* only one nucleus does so, all the rest remaining free. In *Piscicola* one of the multiple cells becomes the ovum, while the others are absorbed. The formation of cells in *Piscicola*, excepting the ovum, would appear to be superfluous, and this part of the process is skipped in *Nephelis*. According to Ludwig the germ-cell in *Piscicola* produces a capsule, which, later, encloses both the ovum and the remaining cells. Whether the egg subsequently became enveloped by a special vitelline membrane was left undecided.

## VI. *The Archiamphiaster.*

With regard to the changes preliminary to cleavage in the egg of *Nephelis*, we have the valuable observations of Bütschli (No. 19) and O. Hertwig (No. 8). In order to see for myself the interesting phenomena described by these authors I have gone over the same ground, and have dis-



covered one very important feature of the archiamphiaster, which these investigators appear to have entirely overlooked, namely, the "spiral aster," which Dr. Mark, of Harvard University, was the first to discover in the egg of *Limax* (No. 23, p. 494, and No. 24).

In the eggs that are found floating in the ovarian fluid the germinal vesicle has usually disappeared, and in its place a bi-stellate figure—the archiamphiaster—is found (fig. 15, Pl. IV). In the centre of each star there is a clear space—the polar area—which in hardened preparations looks finely granular. The astral rays are at first rather weak, but they can be made out, even in the living egg (fig. 19). The interstellate rays appear to meet and form a more or less spindle-shaped area. The spindle rays appear to differ in no essential way from the other radial rays, nor do they, so far as I can see, converge to a point in the centre of either pole of the figure, as Hertwig represents in his figures. At this time the spindle is ill defined, and measures only about  $\cdot 002$  mm. in length. A thickened equatorial zone ("Kernplatte," Strasburger) of the spindle is very doubtfully recognisable.

In eggs examined immediately after deposit (fig. 16) the appearance of the archiamphiaster is markedly different. The polar areas, however, remain without any apparent change. The spindle has acquired a pretty well-defined outline (ca.  $\cdot 03$ — $\cdot 035$  mm. long,  $\cdot 013$  mm. wide). A glance at figs. 15 and 16 will show that in the latter the spindle has not only elongated, but has become decidedly larger. The spindle rays are distinct, and their thickened equatorial zone is unmistakably established. But what are most interesting are the rays of the asters. They are much bolder in appearance than in eggs found floating in the ovarian fluid (fig. 15). They have greatly increased in length, extending almost to the surface, *describing nearly uniform curves*, so that *they have a spiral arrangement*. Fig. 16 shows this spiral arrangement of both stars. Fig. 17 is an optical section through the lower star in fig. 16, seen from above. The rays of the two stars always curve in the same direction. Dr. Mark remarks that "the spiral may be either dextral or sinistral." In all my drawings, of about a dozen different cases, I find the spirals of both asters dextral when the amphiaster is seen from the side. If, however, either aster is viewed from above, the spiral will be sinistral. This may be accounted for by supposing that the rays have a double curvature.

A few minutes after deposit, as the archiamphiaster pushes

its way toward the surface, the rays gradually diminish in length. At the same time the curvature of the rays become less and less conspicuous, until they are almost straight. Fig. 18 is a stage in which the curvature of the rays is yet retained to a slight extent. In fig. 19, which is a fresh specimen, the rays can almost be said to be straight. In this figure it is to be noted that the yolk-granules are present even in the spindle, whereas in *Clepsine* (No. 7, p. 14) the spindle is free entirely from the yolk-spheres. From this fact I am rather inclined to think that the spindle in the case of *Nephelis* is in a medium in no way different from that in which the rays of the stars are situated. Bütschli (No. 19) and some others seem to lay much stress on the spindle. That it consists of the united rays of the two stars accords with my observations, as well as with those of Fol (Nos. 18 and 21), Bobretzky (No. 22), Whitman (No. 7), and Mark (No. 23).

Notwithstanding my special endeavours I have not been able to get transitional stages between the germinal vesicle and the archiamphiaster. But I was slightly successful with later amphiasters directly concerned in the cleavage. In fig. 20 is represented a divided egg, in which the shrunken nucleus of each cleavage sphere is giving rise to two stars. These stars belong to that amphiaster which divides the egg into four. The left-hand sphere is less advanced than the other. On opposite sides of its nucleus will be seen clear spaces, which are the polar areas. In the right-hand sphere these areas are already surrounded by radial rays. Here the upper star will be noticed as being a little apart from the nucleus.

What represents the nucleus of each cleavage sphere in this figure is probably composed of a number of vesicles collected in a group, as others of my preparations of the same stage clearly show. The amphiaster which leads to the first cleavage always gives rise to a group of nuclei in each cleavage sphere, but I believe they do not necessarily coalesce into a single body before they again dissolve away.

Such stages were also observed in the first cleavage amphiaster. Here the male and the female pronucleus ("Eikern") were placed close to each other in the centre of an egg, and the stars were in process of formation on their opposite sides.

These stages of the amphiaster are probably to be regarded as preceding the stage represented by Hertwig's fig. 5 (*Hæmopis*, Pl. I, No. 18).

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A CONTRIBUTION to the MORPHOLOGY of the AMPHINEURA. By  
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IN August, 1881, I was invited by the editor of this Journal to furnish him with a few diagrams concerning the anatomy of the Amphineura, and with a short explanatory text indicating the actual state of our knowledge about this class of animals. Different engagements have obliged me to postpone the fulfilment of this wish till now. I am not sorry for this delay, now that it enables me to mention the latest researches upon *Chiton*, by Mr. A. Sedgwick, which have thrown a welcome light on the difficult subject of the renal organs of the class.

I will limit myself to a very brief statement of what appears to me to be known, surmised, uncertain, or unknown with respect to the following heads:—*a*, integument; *b*, nervous system; *c*, intestine; *d*, circulatory and respiratory apparatus; *e*, reproductive and excretory organs. As it is not my intention to enter into a full discussion of the different views of the several authors and their respective merits, I will merely summarise those statement which appear to me to be the most worthy of credit. A list of the different authors, to which reference is made in the text by means of a different number prefixed to each of them, will, however, be given at the end of this paper.

A. *Classification*.—The systematic arrangement of the AMPHINEURA and the names adopted for the subdivisions are the following:

| <i>Mollusca.</i> |                       |                    |                 |  |
|------------------|-----------------------|--------------------|-----------------|--|
| <i>Class.</i>    | <i>Order.</i>         | <i>Family.</i>     | <i>Genus.</i>   |  |
| Amphineura. . .  | { Solenogastres . . . | { Chætodermata . . | { Chætoderma.   |  |
|                  |                       | { Neomeniæ . . .   | { Neomenia.     |  |
|                  |                       |                    | { Proneomenia.  |  |
|                  |                       |                    | { Chitonellus.  |  |
|                  |                       |                    | { Chiton.       |  |
|                  |                       |                    | { Cryptochiton. |  |
|                  |                       |                    | &c.             |  |

V. Jhering (8) originally regarded the AMPHINEURA as a separate phylum of the Vermes; Spengel (20) afterwards clearly showed that they will henceforth have to be regarded as a class of Molluscs. *Chætoderma* and *Neomenia* were linked together by Gegenbaur under the name of Solenogastres, which is better chosen than v. Jhering's designation of Aplacophora; the latter, moreover, ranks as a class with v. Jhering. The families of



Chaetodermata and Neomeniæ instituted by v. Jhering can be retained. As to the generic names, the manuscript name of *Solenopus*, Sars, which was given to the specimens in the Bergen Museum, but which was never published, and which Korén and Danielssen (11) nevertheless retain for *Neomenia*, Tullberg, will once for all have to be abandoned, when it is remembered that as early as 1826 the name *Solenopus* was pre-occupied in zoology, C. J. Schönherr having in that year assigned it to a genus of Curculionidæ, Coleoptera on p. 268 of his work 'Dispositio methodica,' &c. Up to the present day the genus *Chaetoderma*, Lovén (14), counts one species (*Chaetoderma nitidulum*, Lovén = *Crystallaphrysson nitens*, Möbius); *Proneomenia*, Hubr. (7), one (*P. sluiteri*); *Neomenia*, Tullb. (28), eight, *Neomenia carinata*, Tullberg, *Neomenia affinis*, *dalyelli*, *incrustata*, *margaritacea*, *borealis*, *sarsii* (all Koren and Danielssen's), and *Neomenia gorgonophila*, Kowalevsky. *Neomenia corallophila*, Kowalevsky, has not been described as yet, although it is mentioned in his explanation of plates (13). I agree with v. Jhering in thinking it probable that Koren and Danielssen's species will perhaps come to be reduced in number when these investigators have examined their specimens more in detail; external shape and size must be looked upon as very misleading specific characters in these animals. *Neomenia sarsii* and *N. gorgonophila* will perhaps prove to be *Proneomenias*.

Of the great number of species and genera amongst the Chitones every text-book on conchology can give evidence; it would lead us too far to enter into any details in this respect. Especially of the genus *Chitonellus*, as will be shown below, a more detailed examination of the different species promises to yield interesting results.

B. *Integument*.—In all the AMPHINEURA a thin cellular layer, which rarely appears to exceed one cell in thickness (3, 7, 11, 13, 18, 22), is applied upon the muscular tissue of the body-wall, and fulfils the function of matrix for the integument. In *Chiton* it is also continued upon those membranous portions of the body-wall which are found in the duplicatures containing the shells.

The integument furnished by this matrix is composed of two elements:

a. A cuticular substance of varying thickness (thickest probably in *Proneomenia*).

b. Calcareous elements deposited within this cuticle, and either forming spicules only (*Solenogastres*), or spicules and plates or shells (*Chitones*).

Structures which may to a certain extent be regarded as transitional between the two are the horny or chitinous bases of certain spicules (18), and still more those horny hairs or setæ

which are sometimes developed in the cuticle side by side with the calcareous spicules, and which may in certain species (*Chiton pallasii*) attain a rather considerable size.

The calcareous spicules, both in the Solenogastres and the Chitones, are of very different sizes and shapes (3, 7, 13, 18, 22). In *Proneomenia* they are of the most uniform shape throughout the whole of the integument (7); in *Chiton* they present the greatest degree of diversity (16, 18).

For certain genera it has been proved that the spicules remain attached to the cellular matrix, even when situated high up in the cuticle close to the outer surface, by a string of cellular tissue (7, 18). A cellular capsule encloses their base in *Proneomenia*; in this they find their origin when it still forms part of the subjacent cellular matrix; they appear to be lifted and the string to grow in length, together with the increase in thickness of the cuticle, which pushes them outwards passively. Similar radial outgrowths of the cellular matrix, which, however, appear to be in no direct connection with the spicules, are figured by Kowalevsky for *Neomenia gorgonophila* (13). Very numerous radial hollow tubes in the shells of *Chiton*, first noticed and figured by Marshall (15), are, moreover, filled during life by strings of tissue, which are direct radial prolongations of the cellular matrix,<sup>1</sup> and have great analogy to the funicles above mentioned (7, 18).

The genus *Chitonellus* is characterised by its inconspicuous dorsal shells, calcareous spicules being distributed in a very regular way in the rest of the skin. This genus was long looked upon as representing a reduced stage in comparison with *Chiton*; different details of its organisation (branchiae, foot, &c.) show the inconsistency of this proposition, and of all Chitones it must certainly be looked upon as the more primitive and the more closely related to the Solenogastres (8). A study of the exact structure and growth of its shells is a great desideratum, especially if very young stages of *Chitonellus*, which at present are so exceedingly scarce in zoological collections, are available.

Two words may here be added concerning the foot, which makes its appearance in the Solenogastres as a median ventral folding of the integument, not covered by chitinous cuticle and spicules, but ciliated (4, 7, 13), and extending in *Neomenia* and *Proneomenia* from close behind the mouth down to the anus. In *Chatoderma* (4) it is only developed in the posterior half of

<sup>1</sup> I have been able to examine sections through the decalcified shells of *Chiton* made by Doct. Phil. J. F. van Bemmelen, and have satisfied myself that they show the peculiarities alluded to above very clearly. This gentleman being at present occupied in investigating more fully the integument of the Chitones, I here refrain from further details, for which I refer to his paper, which will be shortly forthcoming.

the body, and I think there is more probability of this representing a reduced than an incipient stage of the foot-fold of the other genera.

In *Chitonellus* the foot is undoubtedly less differentiated (8) than it is in *Chiton*, where it must certainly already be regarded as homologous with the foot of Gasteropods, &c.

c. *Nervous system*.—The most conspicuous feature of the nervous system of the AMPHINEURA is the presence of four longitudinal nerve-trunks, united together into one in front of or above the pharynx. The fact of the presence of nerve-cells, intermixed to a considerable extent with the fibrous nerve-matter along the whole course of these trunks, shows that centralisation has not yet by any means reached its limit in this class, but that the whole of the longitudinal stems may, to a certain extent, be looked upon as representing the central nervous system. An anterior cerebral thickening appears to be more marked in the *Solenogastres* (3, 6, 7, 22) than in the *Chitones* (1, 8, 20). A posterior coalescence of the four longitudinal stems, or of two of them, into a ganglionic swelling situated above the rectum has been demonstrated with certainty in *Chatoderma* (3, 6), *Neomenia* (4), and *Chiton* (8a, 20). In *Proneomenia* there is as yet only probability of its existence (7); it will have to be looked for carefully in the first specimens that come to hand.

The ventral longitudinal stems are united by transverse commissures in *Chiton* (8, 20), *Neomenia* (4, 22), and *Proneomenia* (7). The first of these commissures thus closes a ring round the pharynx, which may be called the œsophageal ring. Ganglionic swellings at the point where the ventral stems commence their backward course, and are united by this first commissure, may be called the infra-œsophageal ganglia. A second, more delicate, nerve-ring round the pharynx has been demonstrated with certainty in *Chiton* (8, 20), *Neomenia* (4), *Proneomenia* (7), and *Chatoderma* (4, 6); it may be termed the sublingual nerve-ring, and carries a ganglionic swelling—the sublingual ganglion.

The transverse ventral (pedal) commissures are placed at regular intervals, and in *Neomenia* (4) and *Proneomenia* (7) have been shown to take their course partly through the ventral longitudinal blood-sinus. In the latter genus smaller ramifications have been seen to take their origin from these commissures. In *Chiton* similar ramifications from the ventral commissures appear to give rise to a plexus-like arrangement of nerve-tissue in the foot (5).

In *Chatoderma* similar transverse ventral commissures between the longitudinal stems, although specially looked for, have not as yet been found, and may with a certain amount of probability,

FIG. 1.

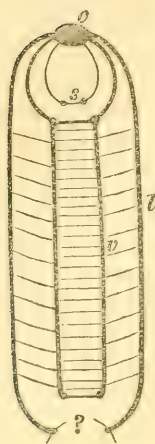


FIG. 2.

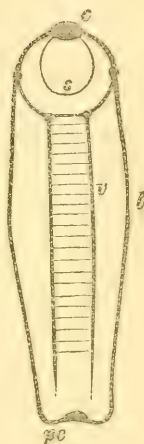


FIG. 3.

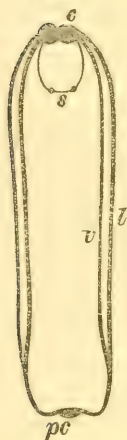


FIG. 4.

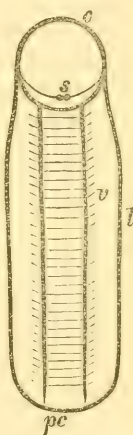


FIG. 1.—Diagram of the nervous system of *Proneomenia*. *c*, cerebrum, *l* lateral, *v*, ventral longitudinal stems; *s*, sublingual commissure. It requires further confirmation<sup>1</sup> whether or not at ? there is a posterior commissure between the two lateral stems.

FIG. 2.—The same of *Neomenia*, copied from Graff (4). Letters as in Fig. 1. *pc*, posterior commissure of the lateral stems.

FIG. 3.—The same of *Chaetoderma*, reconstructed out of the descriptions of Graff (3), and Hansen (6). Letters as in Fig. 2.

FIG. 4.—The same of *Chiton*, copied from Spengel (20). Letters as in Fig. 2.

In all these diagrams the nerves for the head springing from the cerebrum as well as the peripheral branches are omitted.

<sup>1</sup> Later investigations have already shown such a commissure to be present in *Proneomenia*. See the postscript to this article.



indeed, be said to be absent. It remains a matter of some doubt whether in this genus the nerve-ring which is present round the pharynx (4, 6) is comparable to the œsophageal (4) or to the sublingual ring. I hold the latter view to be the more probable. The four longitudinal stems in *Chatoderma* unite, in the hinder part of the body, into two lateral stems, which afterwards coalesce posteriorly in the way above mentioned (3, 6).

It appears to me that the nerve-system of *Chatoderma* must be looked upon, not as a more primitive stage, but as a reduction from an arrangement which was originally more in accordance with that of the other Solenogastres. An additional argument for this view will hereafter be gathered from the structure of the intestine and liver.

Finally, it must not pass unnoticed that in *Proneomenia* the commissural system offers an increase in complication (7), in so far as a series of transverse commissures is present on both sides, uniting the lateral with the ventral longitudinal stems. From these commissures peripheral branches also originate.

In how far these different facts might eventually be grouped, so far as to throw some light on the phylogeny of certain groups of invertebrates, or of the nervous system in general, has already been more fully discussed by me elsewhere, and may here be safely passed over in silence.

D. *Intestine*.—The intestine is simplest in *Neomenia* and *Proneomenia*, somewhat more complicated in *Chatoderma*, and has attained a far higher degree of specialisation in *Chiton*. A muscular pharynx is present both in the Solenogastres and in the Chitones. In *Neomenia* it is capable of partial protrusion (22). It is internally lined by a chitinous cuticle applied upon a layer of columnar cells, and is variously folded. The cavity containing the radula is in open communication with it. In accordance with the size of the radula this cavity is very considerable in the Chitones, very small in *Proneomenia* and *Chatoderma*, apparently absent in *Neomenia*. Shape and situation of the radula of *Chiton* have been fully described by different authors (16, 18a, 21). In 1877, when v. Jhering (8) for the first time defined the AMPHINEURA as a separate group (which, however, he erroneously separated from the Molluscs), he regarded the presence or absence of a radula as one of the chief distinctive characters between the two subdivisions of the Chitones and the Solenogastres (his Placophora and Aplacophora). This distinction broke down when the discovery of *Proneomenia* (7) showed that in the Solenogastres the radula was not always absent, and that there was even more probability in favour of the view that it was undergoing regressive metamorphosis in this group than that it had not yet been started. The chitinous tooth, which in

*Chætoderma* occupies a corresponding position (3, 6), is another argument in favour of this view. I feel very much inclined to look upon it as a stage of simplification of a radular arrangement rather than as a primitive more simple structure, from which, by gradual differentiation, a radula might be derived. It was elsewhere insisted upon (7) that the complicated structure of the radula in *Proneomenia* forbids an interpretation in the latter sense of the link which connects these structures in the different genera of Solenogastres. In *Neomenia* all remains of a radula may safely be said to have disappeared in the specimens that have hitherto been examined; none of the different authors

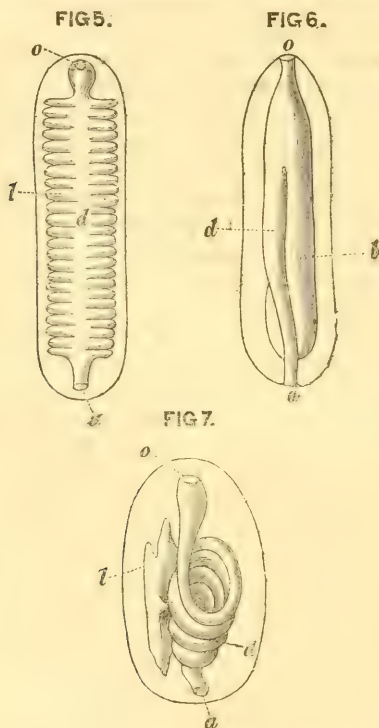


FIG. 5.—Diagram of the digestive tract of *Neomenia* and *Proneomenia*, reconstructed from the descriptions of the different authors (7, 13, 22). *o*, mouth; *a*, anus; *d*, ciliated median portion of the intestine; *l*, lateral caeca, on which the hepatic functions partially devolve.

FIG. 6.—The same of *Chætoderma*; reconstructed after the description of Hansen (6). *o* and *a*, as in Fig. 5; *d*, posterior narrowed portion of the intestine; *l*, liver.

FIG. 7.—The same of *Chiton*. *o* and *a*, as in Fig. 5; *d*, the coiled intestine; *l*, liver.

(4, 7, 10, 22) have at least succeeded in discovering any trace of it.

The slit in the pharynx of *Proneomenia* which gives access to the small radular cæcum, at the same time serves to evacuate the products of two long cylindrical parallel glands, situated under the intestinal epithelium, which converge towards this slit. These glands are regarded as salivary glands (7). They are absent in *Neomenia*; nor has anything of the sort as yet been noticed in *Chaetoderma*. In *Chiton* salivary glands have been described (16), but these would appear to occupy a dorsal position with respect to the pharynx. Whether the latter are nevertheless comparable to those of *Proneomenia*, or whether we shall rather have to look upon the so-called pharyngeal sacs (Schlund-säcke, Middendorf) as the homologue of the tube-shaped glands of the latter genus, will have to be inquired into carefully.

The part of the intestine which follows upon the pharynx is simplest in *Neomenia* and *Proneomenia*. In both genera it occupies the greater part of the space available within the muscular tunic after deduction of the genital gland. It is straight, and on both sides provided with very deep folds, which might be compared to as many (hepatic) cæca. Ciliation has been noticed along the median ventral and dorsal line. The rectum, which posteriorly passes below the pericardium and in the midst of the renal and genital excretory ducts, is narrowed and provided with cilia all over the surface.

In *Chaetoderma* a subdivision of this part of the intestine has taken place, which appears to me to be very well interpreted by Hansen (6), who regards the posterior cæcum-like portion, opening out into the principal cavity, which is terminated by the mouth and anus, as the incipient stage of a separate liver. This stage has been very far surpassed in the Chitones, where the more or less primitive intestinal arrangement of the Solenogastres is replaced by an intestinal tract, which is comparatively narrow, considerably bent upon itself and coiled, and into which a well-separated, dendritically-shaped liver opens. On this head the difference between the two subdivisions of the AMPHINEURA is, as may be seen, considerable.

Only in the Chitons the posterior opening of the rectum opens directly to the exterior; in *Neomenia* and *Proneomenia* its contents are first evacuated, together with those of the excretory and genital organs, into a sort of common cavity or cloaca, the external opening of which serves for both systems. In *Chaetoderma* there is no true cloacal cavity, but the infundibulum into which the rectum and the nephridia open, and in which the branchiæ are placed, nevertheless has a certain analogy with it (fig. 8).

E. *Circulatory and respiratory apparatus.*—In all the genera of AMPHINEURA a heart, situated dorsally, close to the posterior extremity of the body, a median dorsal and a median ventral blood-vessel, are the principal parts of the circulatory apparatus. Paired auricles to the heart are present in the Chitons; their presence in *Neomenia* and *Proneomenia* is not yet put beyond all doubt, but still rendered very probable. The dorsal vessel is the direct anterior continuation of the heart. The latter is situated in a cavity in which blood is never found, which may best be compared to the body-cavity, and to which the inappropriate name of pericardium has been given. It is closed on all sides, with the only exceptions hereafter (p. 228) to be mentioned. The longitudinal vessels open out anteriorly amongst the tissues, the circulation being lacunar for a very great portion (around the intestinal folds, *f. ex*). A part of the lacunar circulation in the foot of the Chitons will most probably have to be regarded as the equivalent of the ventral blood-vessel of the Solenogastres, which similarly lies below the horizontal muscular diaphragm. For details about the circulatory apparatus of *Chiton*, reference may be given to Middendorff's researches (16); suffice it to say that here, as in the Solenogastres, the blood is pumped by the heart out of the gills and driven forwards along the median dorsal vessel towards the genital gland and the head.

As to the respiratory apparatus very different degrees of development are present amongst the AMPHINEURA. In *Proneomenia* special branchiæ were vainly sought for, and if a tuft of hollow threads in one of the folds of the pharynx must not be looked upon as such—functionally at least—we are forced to the conclusion that respiration takes place all along the wall of the intestine and the foot, and perhaps more especially in the rectum.

Both in *Neomenia* and *Chætoderma* retractile branchiæ have been demonstrated at the posterior extremity of the body. They are tuft-like in the former (11), distinctly paired in the latter genus (6), where the anal opening lies between them.

In *Chitonellus* they are no longer paired, but are numerous and stretch between the foot and mantle, to the right and left of the anus, about as far as half way along the body, each branchial process having to be looked upon as a unit in comparison to the gills of the Prosobranchia (2, 20). In the genus *Chiton* the lateral branchial series are even extended further forwards, reaching as far as the head. Hand in hand with this marches a complication in the circulatory apparatus.

F. *Excretory and generative organs.*—This apparatus and its different modifications in the various genera and species of the AMPHINEURA perhaps requires more than any other renewed and careful investigation. A few years ago the confusion was even



far more considerable ; but still, notwithstanding the light which has been thrown upon this subject by the researches of Hansen, Sedgwick, and others, different details as yet only repose upon insufficient evidence (strengthened though it may be by ingenious speculations), and should be re-examined whenever specimens of these very rare species are available.

In the following short account I will try to give a fair valuation of the statements of the different authors, at the same time endeavouring to hold myself free from any preconceived opinions on the subject.

If we except Graff's account of the genital organs and the oogenesis of *Chatoderma* (3), which, however, has afterwards been criticised and corrected by Hansen (6), all authors unanimously place the genital gland of the different genera of Amphineura in the median line of the dorsum, immediately below the integument, and with only the median dorsal blood-vessel superior to it. The genital gland stretches throughout the greater part of the length of the animal, is more or less symmetrical, and was found in *Proneomenia* (7) to be regularly split up ventrally into two halves, and to have a multilobate appearance. The sexes are separate in the Chitons (10, 19) and in *Chatoderma* (6), whereas *Neomenia* and *Proneomenia* appear to be hermaphrodite (7, 11). The latter genus, however, has as yet never been examined in the fresh state.

With respect to the way along which, in the Chitones, the genital products travel outwards, certain divergent opinions have to be recorded in succession. According to the researches of Cuvier (1), Middendorf (16), von Jhering (10), and Sedgwick (19), there are two ducts, a left and a right one, which leave the genital gland on the dorsal surface, close to its posterior extremity, and strike for the branchial furrow, into which they open between a pair of the posterior branchiæ. This passage is coiled in the female, straight in the male (19). I have myself been able in sections to further confirm the presence of the same arrangement in *Chiton marginatus*. Dall (2) has noticed certain different modes of egress for the genital products, and mentions the presence, in some species, of a simple genital pore, in others of a fenestra, *i.e.* of a slit which is divided by bridges of tissue into from two to seven openings. Not finding an oviduct in the latter case, he is inclined to suppose that the eggs are set free in the body-cavity, and from thence pass outwards through these fenestræ. These observations are in great need of further confirmation.

Before passing on to the genital apparatus of the Solenogastres I hold it to be appropriate to mention the renal or excretory apparatus of the Chitones, as these two systems, which

FIG 8.

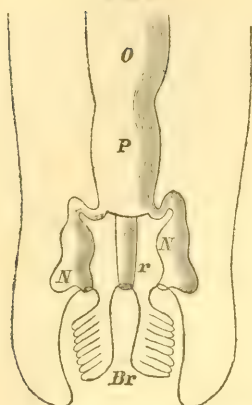


FIG 9.

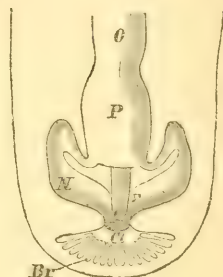


FIG. 8.—Diagram of the genital and excretory system of *Chetoderma*, seen from above. Reconstructed after the description and figures of Hansen (6). *O*, genital gland; *P*, pericardium; *N*, Nephridia; *r*, rectum; *Br*, branchiae, situated with the openings of *N* and *r* in the infundibulum.

FIG. 9.—The same of *Neomenia carinata*. *O*, *P*, *N*, *r*, and *Br*, as in Fig. 8. *Cl*, cloacal cavity, into which *r* and *N* open out.

In this figure and in the foregoing the exact mode of communication between *O* and *P* could not be represented, this having not yet been satisfactorily settled.

FIG 10.

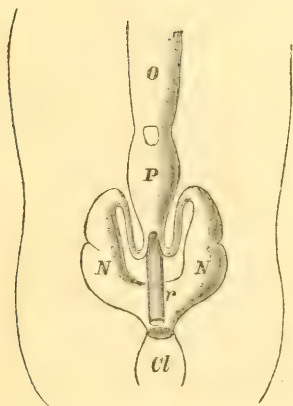


FIG 11.

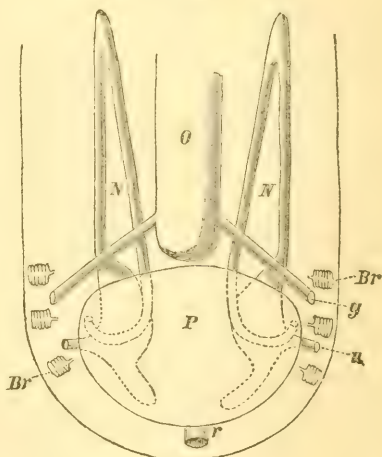


FIG. 10.—The same of *Proneomenia*. Letters as in Fig. 9.

FIG. 11.—The same of *Chiton*. For the greater part copied from Sedgwick (19). Letters as in Fig. 9. *g*, genital opening; *u*, exterior opening of Nephridia.

are separate in this subdivision of the AMPHINEURA, appear to be in close connection with each other in the Solenogastres.

Our knowledge of the nephridia of *Chiton* has only very lately been thoroughly established by Sedgwick (19). They are double, and open into the pericardium at one end, into the pallial groove between the branchiæ at the other. From the pericardium the duct bends forwards towards the head, makes a very sharp turn backwards again, enlarges to a kind of bladder, from whence a short duct leads outwards at a very short distance behind the exterior opening of the genital ducts. Numerous fine branches and delicate ramifications (not represented in the woodcut) are in direct communication with these ducts, and form the mass of the renal organs.<sup>1</sup>

It needs no further inquiry whether the kidney, with a single, posterior, median opening, such as it was described by v. Jhering (10), is really to be found in any existing species of *Chiton*, as Dr. Brock of Göttingen kindly writes to tell me that v. Jhering has lately withdrawn this view as reposing on an erroneous observation, and has been convinced of the presence of lateral renal openings (prior to Sedgwick's exhaustive researches).

We have now to consider the other subdivision of the AMPHINEURA, the Solenogastres. A direct communication between the ovary and the pericardium has been demonstrated in *Pro-neomenia* (7) and *Chætoderma* (6). In *Neomenia carinata* its presence is probable in the highest degree (22), although neither here nor in *Chætoderma* have the ducts been separately made out.

In the second place the different genera of Solenogastres are provided with a system of ducts and passages by which the pericardium communicates with the exterior. These ducts, or parts of them, are considered by the different authors on grounds for which we refer to the original papers (6, 7) as renal organs. There can be no serious doubt about their homology with those of the Chitones. And so the Solenogastres exemplify a primitive stage, in which the pericardium (body-cavity) receives the oviducts, on the one hand, and on the other communicates with the exterior by means of the nephridia. This latter communication persists in a very large number of Molluscs; the former, however, has been given up, but it is exceedingly instructive and remarkable that (as a remnant of it) in the most primitive genera of different classes of Molluscs (*Dentalium*, *Patella*, *Fis-*

<sup>1</sup> I may here add that these recent observations of Sedgwick's have been fully confirmed by Mr. J. F. van Bemmelen, who dissected a large species of *Chiton* from the Indian Ocean, and showed me his preparations, which I found to correspond in all important respects with Sedgwick's account.

*surella*, *Spondylus*) there is a direct discharge of the genital products into the cavity of the kidney. In the next stage the genital and urinary ducts open out upon the same papilla (*Pinna*, *Mytilus*); in the remaining majority the separation has become even more complete, and the external openings more distant; the primitive arrangement, in which ovary, pericardium, and nephridium lead into one another in an unbroken order of succession, being retained in the Solenogastres alone.

A reserve has to be made with respect to the male genital products of *Neomenia carinata*. These are evacuated along separate lateral ducts, provided with calcareous penes, and connected, according to the observations of Koren and Danielssen (11)—which have not yet been repeated—by separate vasa deferentia with the hermaphroditic gland.

Similar penes are absent in *Proneomenia*, and although only two specimens have as yet been examined, it seems improbable that they will afterwards be detected in others, as the specimens under observation appeared to be true hermaphrodites, and not simply females, both ova and spermatozoa occurring in parts of the ducts (7). Nor has a similar arrangement been observed in *Chætoderma* or in the Chitones.

It will be apparent from the foregoing, that further careful observations on the male genital ducts of *Neomenia carinata*, which Koren and Danielssen confess to have only imperfectly made out (11), as well as on the exact mode of communication between the genital gland and the pericardium in this genus and in *Chætoderma*, are very much wanted. Furthermore, a comparative histology of the renal organs, marked *N* in the woodcuts, will have to be made. It must be remarked that in *Chætoderma* (fig. 8) these organs open to the exterior separately, whereas they coalesce into a single duct, with only one median opening, in *Proneomenia* and *Neomenia* (figs. 9 and 10).

The different accessory glands of the genital apparatus, which have been described as such (7, 11), are here passed over in silence, because in the present stage of our knowledge a comparison of these with each other would be premature. The examination of specimens in the fresh state will alone enable us to form a sound judgment on these points.

Nor are structures, such as the presumed byssus-like glands of *Proneomenia* and *Neomenia* (7), the foot gland, &c., here taken further notice of, because our knowledge is not yet far enough advanced to admit of a fruitful comparison.

In order to facilitate comparison of the woodcuts (1—11) inserted in this paper, with the different illustrations given by the several authors on the Solenogastres of the genital and excretory organs, &c., of these animals, I have added the following key to



the figures of Graff (3), Hansen (6), Tullberg (22), and myself (7).

|                                                                           | Graff<br>(3).                                                               | Hansen.<br>(6)                                       | Tullberg<br>(22).                                           | Hubrecht<br>(7).                                                                                                                   |
|---------------------------------------------------------------------------|-----------------------------------------------------------------------------|------------------------------------------------------|-------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|
| The genital gland . .                                                     | <i>u</i> , fig. 14                                                          | <i>gk</i> , Pl. III and IV                           | <i>u</i> , <i>v</i> , <i>w</i> , Pl. I                      | <i>u</i> <i>g</i> , Pl. III and IV,<br>fig. 51.                                                                                    |
| The communication between this and the pericardium                        | ...                                                                         | fig. 3, Pl. IV                                       | ...                                                         | fig. 38, 46, and 47.                                                                                                               |
| The pericardium . .                                                       | ...                                                                         | <i>pc</i> , Pl. IV and V                             | <i>x</i> , fig. 6                                           | <i>Per</i> , fig. 39; <i>P</i> , fig. 46.                                                                                          |
| The nephridium connecting the cavity of the pericardium with the exterior | <i>Ks</i> (interpreted as "Kiemen-säcke" = branchial sacs), figs. 15 and 16 | <i>sk</i> , Pl. IV and V                             | <i>q</i> , <i>r</i> , fig. 6; <i>a</i> , <i>b</i> , fig. 29 | <i>d</i> , <i>A</i> , <i>R</i> , <i>d</i> <sup>1</sup> , <i>A</i> <sup>1</sup> , <i>R</i> <sup>1</sup> , figs. 32, 38, 39, and 46. |
| The dorsal blood-vessel                                                   | <i>el</i> (interpreted as oviduct), figs. 4, 5, 6, 7, 12, 13, and 32        | <i>vd</i> , Pl. I, II, and III; <i>cr</i> , IV and V | ...                                                         | <i>ds</i> , Pl. I, III, and IV.                                                                                                    |
| The ventral blood-vessel                                                  | <i>b</i> , figs. 4, 5, and 6                                                | <i>vv</i> , Pl. I—IV                                 | <i>u</i> , figs. 6 and 7                                    | <i>vs</i> , Pl. I—III.                                                                                                             |
| The liver . . . .                                                         | <i>zb</i> , <i>bgl</i> , fig. 14                                            | <i>l</i> , Pl. III and IV                            |                                                             | <i>tf</i> , fig. 14; <i>f</i> <sup>1</sup> , fig. 48.                                                                              |

Of the figures given by Möbius (17), 11 represents one of the branchia; whereas 10 either stands for the œsophagus (cf. Hansen, Pl. I, fig. 2, *o*) or for the liver (cf. id., Pl. IV, figs. 1 and 3, *l*) of *Chaetoderma*.

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- (5) *B. Haller*, "Ueber das Nervensystem und Mundepithel niederer Gastropoden," 'Zoologischer Anzeiger,' iv, 1881, p. 92.
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- (7) *A. A. W. Hubrecht*, "*Proneomenia sluiteri*, gen. et sp. n., with remarks upon the Anatomy and Histology of the Amphineura," 'Niederländisches Archiv für Zoologie,' Supplement Band, 1881.
- (8) *H. von Jhering*, 'Vergleichende Anatomie des Nervensystems und Phylogenie der Mollusken,' Leipzig, 1877.
- (8a) *H. von Jhering*, "Beiträge zur Kenntniss des Nervensystems der Amphineuren und Arthrocochliden," 'Morphologisches Jahrbuch,' Band iii, p. 156.
- (9) *H. von Jhering*, "Bemerkungen über *Neomenia* und über die Amphineuren im Allgemeinen," 'Morphologisches Jahrbuch,' 1878<sup>1</sup> iv, p. 147.
- (10) *H. von Jhering*, "Beiträge zur Kenntniss der Anatomie von Chiton," 'Morphologisches Jahrbuch,' iv, 1878, p. 128.
- (11) *I. Korén* and *D. C. Danielssen*, "Beskrivelse over Nye Arter, hørende til Slægten *Solenopus*," 'Archiv for Mathematik og Naturvidenskab,' Christiania, 1877 (translated in the 'Ann. and Mag. of Nat. Hist,' ser. 5, vol. iii, p. 321).
- (12) *A. Kowalevsky*, "Ueber der Bau und die Lebensweise von *Neomenia gorgonophila*," n. sp., "Verhandlungen der Zoologischen Section der vi. Versammlung russischer Naturforscher und Aerzte," 'Zoologischer Anzeiger,' iii, p. 190.
- (13) *A. Kowalevsky*, '*Neomenia gorgonophila*' (published in Russian). Moskau, 1881, 4<sup>o</sup>.
- (14) *S. Lovén*, 'Öfversigt af Kongl. Vetensk. Akademiens Förhandlingar,' Stockholm, 1844, p. 116, Tab. ii.
- (15) *W. Marshall*, "Note sur l'histoire naturelle des Chitons," 'Archives Néerlandaises des Sciences exactes et naturelles,' vol. iv, 1869, p. 328.
- (16) *A. Th. Middendorff*, *Malaco-zoologia rossica*, I "Beschreibung und Anatomie neuer Chitonon," &c., 'Mémoires de l'Acad. Imp. des Sc. de St. Pétersb.,' 6<sup>me</sup> série sc. nat., t. vi, 1849, p. 67.
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- (18) *J. Reincke*, "Beiträge zur Bildungsgeschichte der Stacheln im Mantelrande der Chitonon," 'Zeitschrift für wissenschaftliche Zoologie,' vol. 18, p. 805.

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*Postscript.*—While the foregoing paper was passing through the press an article appeared in No. 103 of the 'Zoologischer Anzeiger,' written by Kowalevsky and Marion, in which anatomical details are furnished concerning certain *Neomenia*-like animals which these authors have obtained at Marseilles, and which is announced as being preliminary to a more elaborate paper with accompanying illustrations.

The paper is of a very revolutionary tendency, proposing no less than to look upon Tullberg's description of *Neomenia carinata* as having been erroneously inverted. Tullberg is said to have described (1) as *posterior* "lateral glands" what are in reality *anterior* salivary glands; (2), as calcareous penes what is in reality a radula; (3), as supra-rectal "egg-bag" what is an intestinal diverticulum above the pharynx; (4) as branchiæ alongside of the anus what are pharyngeal fringes; (5), as a protrusible pharynx what are no less than oviducts and a uterus, with their respective internal intercommunicating cavities.

In the following number of the 'Zoologischer Anzeiger,' I exposed the reasons upon which my utter disbelief in the hypothesis of these two distinguished authors was founded. I will not enter in detail into this controversy, nor give a translation of my refutations in the 'Zoologischer Anzeiger,' as I have reason to suppose this periodical within easy reach of any reader of this article. It may suffice to refer the reader to the comparisons drawn in the foregoing pages, and to remind him that personal investigation of *Neomenia carinata* (which was neglected by Kowalevsky and Marion) has enabled me to confirm the results of Tullberg's observations—as has already been done before by Koren and Danielssen, and by Graff—in all important points, and thus to conclude (1) that the lateral glands are not salivary glands; (2), that calcareous penes are present and not to be confounded with a radula; (3) that Tullberg's "egg-bag" is the pericardium, and not an alimentary diverticulum above the pharynx; (4) that a posterior tuft of branchiæ is present; (5)

that the protrusible pharynx corresponds very well to the figure which Tullberg gives to this region.

Finally, I suggested that the animals with which Kowalevsky and Marion have been experimenting at Marseilles must be referred to *Proneomenia* rather than to *Neomenia*, especially because the results of their careful anatomy fully confirm the different statements made in the foregoing pages concerning the first-named genus.<sup>1</sup>

<sup>1</sup> Simultaneously with this proof-sheet I receive a letter from *Professor A. F. Marion*, at Marseilles, in which he authorises me to say that he has changed his mind with respect to the Marseilles specimens, in so far as he agrees with me in regarding the genera *Neomenia* and *Proneomenia* as perfectly distinct, and his specimens as certainly belonging to the latter genus. This being the case we may henceforth declare *Proneomenia* to be in the possession of a posterior commissure of the lateral nerve-trunks, and the point of interrogation in fig. 1, on p. 221, may safely disappear; as Professor Marion moreover writes to tell me that one of his Marseilles species of PRONEOMENIA is in the possession of such a commissure, entirely corresponding to that of the other AMPHINEURA.

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*On the CHLOROPHYLL-CORPUSCLES and AMYLOID DEPOSITS of SPONGILLA and HYDRA.* By E. RAY LANKESTER, M.A., F.R.S., Jodrell Professor of Zoology in University College, London. (With Plate XX.)

QUITE recently (November, 1881) Dr. Karl Brandt<sup>1</sup> has adduced reasons for considering the green-coloured corpuscles which occur in the cells of *Spongilla fluviatilis* and of *Hydra viridis* as parasitic Algæ, and has given to those occurring in the former the name "*Zoochlorella parasitica*," to those occurring in the latter the name "*Zoochlorella conductrix*."

Professor Semper, of Wurzburg, had previously expressed a similar opinion as to the nature of these green-coloured corpuscles. In his remarkable volume, '*Animal Life*' (International Scientific Series, p. 73), Semper brings forward facts which he thinks "may soon require us, if we find true chlorophyll in animal tissues, to recognise in its presence a singular and interesting case, either of parasiticism or of the community of two organisms so different, as an animal with true tissues and organs and a one-celled plant." Semper is led to this view by the analogy of Lichens, the duplex nature of which was demonstrated by Schwendener. He is further influenced by and cites Cienkowsky's observations and conclusions as to the "yellow corpuscles" of Radiolarians. But the value of Semper's judgment in the matter is very much diminished by the fact that he expressly declares that the chlorophyll bodies of plants do not divide and multiply (p. 74). Upon this erroneous conception of the chlorophyll bodies of plants Semper bases his argument as to the probability of the green corpuscles found in animals being independent Algæ, since in some cases these green corpuscles have been observed to multiply by transverse division. It is, however, well known that the chlorophyll bodies of green plants also multiply by transverse division.

The probability in favour of the independent algal nature of the green corpuscles of *Spongilla* and *Hydra* was largely increased between the publication of Professor Semper's conclusions and the memoir of Dr. Karl Brandt by the observations of the Hertwigs on the yellow corpuscles of Radiolarians and on yellow corpuscles, which occur abundantly in the endoderm of Anthozoa (*Actiniæ*). The Hert-

<sup>1</sup> 'Sitzungsbericht der Gesellsch. Naturforsch. Freunde zu Berlin,' No. 9, 1881.

wigs confirmed the observations of Cienkowsky as to the independent nature of the Radiolarian yellow corpuscles, and held them to be parasitic. They also demonstrated that the yellow corpuscles of Anthozoa had the structure and properties of unicellular Algæ, and were inclined to regard them also as parasitic.

The term "symbiosis," introduced I believe originally to express the relation of the green algal gonidia of Lichens and the associated colourless Inophyte, has been recently extended with great effect to include the relationship of the Radiolarians to their yellow cells, and also of Anthozoa to their yellow cells.

Whilst there appears to be very nearly sufficient ground for accepting the conclusion thus formulated in regard to the Radiolarians and the Anthozoa, I shall endeavour to show in the following pages that there is no evidence to justify the extension of the doctrine of symbiosis to Spongilla and Hydra, as advocated by Professor Semper and Dr. Brandt. It appears to me, on the contrary, that an examination of the green-coloured corpuscles of Spongilla and Hydra demonstrates those corpuscles to be similar in nature to the "chlorophyll bodies" of green plants, and that there is no more reason to regard them as symbiotic Algæ than there is to regard the green corpuscles in the leaf of a buttercup as such. The results of my examination of the green corpuscles of Spongilla and Hydra, which have been made at intervals during the past seven years, are given below. I have already in this Journal (vol. xiv, p. 400, 1874) published a note relative to the chlorophyll-corpuscles of Spongilla. It is, perhaps, worth pointing out, that in advocating the view that what I venture to consider as a normal product of the living activity of animal cells is in reality a parasite, Dr. Brandt is exactly in the converse position to that occupied by Dr. Gaule, of Leipzig, whose opinion that the parasitic Drepanidium phase of a Gregarina is a normal product of the living activity of the blood-corpuscles and other cells of the Frog, I controverted in the last number of this Journal.

**Nature of chlorophyll.**—The green colouring matter which gives its characteristic tint to foliage is not a pure chemical substance, but a somewhat variable mixture of coloured substances, none of which have yet been properly isolated and characterised by the chemist. Hence there is no little difficulty encountered when we attempt to definitely and satisfactorily identify the green-coloured substances which appear in lower plants and in some animals with the

green-coloured substance which tints the "chlorophyll bodies" of higher plants.

The introduction of the spectroscope, as a means of identifying coloured substances in solution, has resulted in the discovery of the complex nature of "chlorophyll." Starting from the important observations of Stokes, followed by those of Kraus and other continental observers, we come to the latest and most complete investigation of "chlorophyll," which is that of Mr. Sorby.

Sorby had the advantage of having made an extensive study of vegetable and other colouring matters by the aid of his beautiful micro-spectroscope and the systematic use of chemical reagents. His method of investigation is primarily based upon that of Stokes. He distinguishes, in relation to the green leaves of the higher plants, colouring matters soluble in water (erythrophyll and chrysotannin group) and those soluble in absolute alcohol and often in carbon bisulphide (chlorophyll group, xanthophyll group, and lichnoxanthine group). The green substances are those known as the "chlorophyll group," and occur in the so-called chlorophyll bodies in association with the yellow substances of the xanthophyll and lichnoxanthine groups. The chlorophylls are distinguished as blue and yellow chlorophyll, found in higher plants, and chlorofucine, which occurs in association with the two preceding, in certain Algæ. Blue chlorophyll is separated from yellow chlorophyll by its greater solubility in certain media. The absorption spectra of these and of chlorofucine present bands differing in position; they are in each case precisely fixed and represented in a diagram by Sorby, as are the absorption bands of each species of the xanthophyll and lichnoxanthine groups. Upon all these substances acids and alkalies have certain definite effects, in some of them profoundly modifying the absorption spectrum, and clearly giving rise to new chemical compounds; whilst in other cases a slight modification only is produced by such reagents. According to Sorby, various observers previous to himself had, in studying the absorption spectrum of the alcoholic extract of a green leaf, considered the seven-banded spectrum due to a mixture of blue and yellow chlorophyll, together with the products of the action of acids upon those substances—as characterising one simple substance—the so-called "chlorophyll." According to Sorby, such a mixture occurs ordinarily in alcohol when allowed to act upon a green leaf. Various analytical processes must be used to separate the various bodies thus occurring in company, and precautions adopted to prevent



the modifying effects of acids in solution. The deep absorption band in the dark red, which is seen even in weak solutions of green colouring matters spoken of by other observers as "chlorophyll" or "chlorophylloid substance," is, according to Sorby, present in a slightly varied position in the case of blue chlorophyll, in that of yellow chlorophyll, and in that of chlorofucine, and also in a totally distinct substance called Bonelleine. Hence no very precise conclusion can be based upon the occurrence alone of this strong absorption band in the red (as, for instance, had been done by myself 7 in the case of Hydra, Bonellia, Idotea, and Chætopherus).<sup>1</sup>

Moreover, the fine red fluorescence which is seen in an alcoholic solution of leaf green is exhibited, according to Sorby, equally by blue and yellow chlorophyll and by chlorofucine, and, as we now know, by Bonnelleine and by Moseley's Pentacrinin, the red rays having, however, in each case a different and characteristic refrangibility.

Thus, it appears that the investigation of the claims of any given greenish coloured pigment to be regarded as "chlorophyll" is by no means a simple matter. Supposing the pigment to be soluble in alcohol, we still have to ascertain which of Sorby's three groups (chlorophylls, xanthophylls, lichnoxanthines) are present, and which of each of the species distinguished by him within those groups.

In order to do this we have to rely on :

1st. Variations in degree of solubility in such media as alcohol, ether, benzine, carbon bisulphide.

2nd. Absorption spectra of the series of solutions obtained.

3rd. Fluorescence and spectrum of the fluorescent light of such solutions.

4th. Reactions of the solutions with acids, alkalies, and oxidising and reducing agents, which give rise to new compounds or change the spectra characteristically.

There are, however, yet to be mentioned two categories of phenomena in relation to the chlorophyll bodies of green plants which comprise data of a nature to assist us in judging of the similarity or dissimilarity of the green pigments of animals compared with that of the chlorophyll bodies. These are, firstly, the *physiological* activities associated with the chlorophyll bodies of plants; and, secondly, the *morphological* features of these bodies.

<sup>1</sup> It is, however, very probable that blue chlorophyll and yellow chlorophyll, as well as the xanthophylls and lichnoxanthines, are *successive physiological stages* of metamorphosis of one (colourless?) original mother-substance, since they appear always to be found associated with one another.



If we find in an organism physiological processes associated with the presence of a green pigment, which processes are identical with those associated with the presence of the green pigment occurring in the chlorophyll bodies of plants, we have so far a certain amount of evidence in favour of the identity of the green pigment in the two cases. And, again, if we find that the green pigment in an organism occurs in corpuscles which are morphologically similar to the chlorophyll bodies of plants, we have so far evidence in favour of the identity of the green pigment in the two cases.

This evidence, both as regards the physiological and the morphological phenomena, will acquire weight in proportion as those phenomena are elaborate or of a peculiar nature.

It has long been known that whatever may be the part actually taken by the green pigment of the chlorophyll bodies of plants in the process, the presence of this green pigment is constantly associated with the decomposition of  $\text{CO}_2$  by the green parts of plants in sunlight, accompanied by the liberation of oxygen gas, and frequently with the formation in the green-coloured chlorophyll-corpuscles of starch. And conversely, it is true that these chemical processes are not known to occur except in the presence of the peculiar complex of coloured substances which give to the chlorophyll bodies their green colour. The recent discussion by Pringsheim of the actual part played by the green pigment in this physiological phenomenon is reported on at some length in another part of this Journal. It is sufficient for our present purpose that the process is constantly and exclusively associated with the presence of the green-coloured chlorophyll bodies. If this very remarkable and peculiar physiological process can be shown to occur in the green-coloured parts of other organisms, we have ground for supposing the green-coloured parts to be of the same nature as the chlorophyll bodies of plants. This line of evidence we may therefore add to the four already established by Sorby, and distinguish it as the *physiological evidence*.

Similarly, the so-called chlorophyll of the chlorophyll bodies of green plants has been shown to occur very usually as a pigment impregnating certain definitely shaped corpuscles which are lodged in and form part of the protoplasm of the vegetable cell. With exceptions amongst the lower Algæ, these corpuscles are spherical bodies, which are distinguished from the protoplasm in which they lie by their differing density. They appear to consist of albuminoids and are not diminished in bulk when the green

pigment is completely dissolved away from them so as to leave them colourless. They have not the nature of "cells within cells," for they possess nothing within them comparable to a cell-nucleus. Rather they have been compared each to a nucleus. They are devoid of special wall or capsule, and have been observed in a state of transverse division, from which it is inferred that they may multiply by fusion, just as nuclei may multiply in multinuclear cells. As to the internal structure of the colourless basis of a vegetable chlorophyll-corpuscle, there appears to be less known than might be anticipated. The central portion appears to be the seat of the formation of starch in certain cases, and this implies a differentiation of superficial and central substance. The green pigment is sometimes observed to impregnate only the superficial layer of the corpuscle so as to form a kind of green-coloured shell enclosing colourless contents (starch, &c.). This is important for the comparison with the supposed chlorophyll-corpuscles of animal tissues.

The development of the chlorophyll bodies of plants and the effect upon them of physical agents is at present receiving renewed attention. It has been observed that they are preceded by colourless corpuscles which under the influence of sunlight develop the green pigment in themselves. And it has also been observed that in certain saprophytes allied to green plants, but themselves devoid of green pigment, colourless corpuscles are present which appear to represent the chlorophyll bodies, and these acquire a green colour when acted upon by chemical agents (sulphuric acid).

If we find green-coloured corpuscles present in the cells of an organism which exhibit the form and structure just detailed, we have evidence so far in favour of such corpuscles being of a nature identical with that of the chlorophyll bodies of plants.

This line of evidence in relation to the inquiry into the supposed existence of chlorophyll in animals we may add as a sixth to those already enumerated, calling it the *morphological evidence*.

It may be added here that over and above the broad physiological and morphological characters of the chlorophyll bodies of green plants which have been above cited, there are other morphological and physiological characters less important or less familiar, which may of course be made use of in a comparison between these bodies and similar bodies occurring in animals.

Definite proof of the occurrence of chlorophyll in an

animal.—There is at present only one example of an animal in which the frequent presence of that complex association of pigmented bodies termed “chlorophyll” has been fully demonstrated.

This animal is the freshwater sponge (*Spongilla fluviatilis*), which often exhibits a brilliant green colouration of its substance, though colourless or pale flesh-coloured growths of it are very abundant under some circumstances.

Before Sorby had made his investigations on chlorophyll, I endeavoured to confirm the opinion which was current as to the probable identity of the green pigment of some lower animals with the “chlorophyll” of plants, by the application of Sorby’s micro-spectroscope and a comparison of the bands of absorption afforded by the pigments in question with those shown by a solution of leaf green in alcohol.

I obtained in the case of *Hydra* and in the Crustacean *Idotea* a dark band identical with that of some solutions of leaf green. In the case of *Spongilla*, I was able to procure larger quantities of the colouring matter, and also in the case of *Chætopterus* (from the epithelium of the dark lobes of the alimentary canal) and in that of *Bonellia*. I was led to the conclusion that the green colour of *Spongilla* was allied to but not identical with “chlorophyll,” and that the dark green pigment in *Chætopterus* and in *Bonellia* was actually “chlorophyll.”

My results were unsatisfactory, owing to the fact that in the first set of cases (*Hydra*, &c.) it was not possible to obtain a sufficient quantity of the colouring matter to operate upon; and in the second set of cases my results were equally faulty, owing to the fact that there was not a proper standard of comparison in the knowledge which then existed as to leaf green or vegetable chlorophyll itself.

As soon as Mr. Sorby’s researches on leaf green were published, I persuaded him to undertake the investigation of the two reputed cases of occurrence of chlorophyll in animals in which I could promise him a sufficient supply of material. These were *Spongilla fluviatilis* and *Bonellia viridis*. I supplied Mr. Sorby with a quantity of green-coloured *Spongilla*, and with a strong solution in alcohol of the green pigment of *Bonellia*. Mr. Sorby’s researches on the green pigments of these two animals were published in this journal (vol. xv, p. 47 and p. 166), and constitute the only adequate investigation of reputed chlorophyll substances from animals.

They resulted in the complete demonstration of the identity of the green pigment present in *Spongilla* with that



occurring in the chlorophyll bodies of normal green plants, whilst what had been taken by Schenk as well as by myself for chlorophyll in *Bonellia*, was shown to be distinct from any of the pigments of the chlorophyll group, although much resembling in its seven-banded absorption spectrum and its red fluorescence certain combinations of that group.

To the pigment of *Bonellia* Mr. Sorby gave the name *Bonelleine*. The physiological evidence of correspondence between this substance and the green pigment of plants has recently been sought for by Mr. Geddes with a negative result. He has found that *Bonellia* does *not* in the living state when exposed to sunlight decompose carbonic acid with liberation of oxygen gas.

Mr. Sorby established the identity of the green pigment of *Spongilla* with that of the chlorophyll bodies of higher plants by obtaining from it and identifying with the spectroscope the following substances soluble in carbon bisulphide, viz. blue chlorophyll, yellow chlorophyll, orange xanthophyll, xanthophyll, yellow xanthophyll, and lichnoxanthine. He also obtained a small quantity of a yellow substance soluble in water, differing from the chrysophyll of higher plants. The substances found in *Spongilla* soluble in carbon bisulphide are precisely those which are present in the ordinary chlorophyll bodies of green plants, in the same association, but their proportions are a little different in the two cases, according to Mr. Sorby.

No *physiological evidence* as to the nature of the green substance present in *Spongilla* has been brought forward by any observer, and I have not made any experiments in that direction. It is still not known whether the green parts of *Spongilla* decompose carbonic acid and liberate oxygen in the presence of sunlight.

The *morphological evidence* relating to it has also until quite recently been neglected. An account of my observations in this respect will be found below.

Thus, then, *Spongilla* remains the only animal in which the presence of a green pigment identical with that of the chlorophyll-bodies of plants has been definitely established by chemical and spectroscopical investigation. The full corroboration of the identity by physiological and morphological evidence is still wanting.

**Pigment of *Chætopterus* and *Pentacrinus*.**—It would be easy to obtain the chlorophyll-like pigment of *Chætopterus* in large quantity, and I anticipate that it would be found to be similar to if not identical with *Bonelleine*.

In this connection I would draw attention to the interest-



ing green pigment (purple when acidified) obtained by Mr. Moseley in great quantity from species of *Pentacrinus* dredged by H.M.S. "Challenger." The banded absorption spectrum of alcoholic solutions of this substance is figured by Mr. Moseley in vol. xvii of this Journal, in a memoir containing a great number of very important observations, which appear to have been overlooked by those who have recently been studying the characters of animal pigments.

Chlorophylloid pigment of *Hydra viridis*.—As to the chlorophyll-like substance of *Hydra viridis*, it is to be observed that there is very great difficulty in obtaining such a quantity of it as will suffice for complete spectroscopic study with reagents such as Sorby has carried out in the case of *Spongilla*. Hence the general indication of chlorophyll-like substances afforded by the single strong absorption band in the red, acquires in this case importance. Still more important is the physiological and morphological evidence with regard to it which will be detailed below, and suffices to render the identity of the pigment of the green corpuscles of *Hydra* with that of the chlorophyll bodies of plants highly probable.

Physiological evidence as to the occurrence of chlorophyll in animals.—At present the attempt to prove the identity of a green pigment occurring in an animal with that of the chlorophyll bodies of plants, by proving the green tissues of the animal to be capable of decomposing  $\text{CO}_2$  in sunlight with liberation of oxygen gas, as the green tissues of plants are known to do, has only been made with success in one case.

Mr. Geddes has shown that a green marine Planarian worm (*Convoluta Schultzei*), when exposed to sunlight in water containing  $\text{CO}_2$  in solution, evolves an appreciable amount of oxygen gas. Supposing that the green pigment of the chlorophyll bodies were itself the agent of the decomposition which is associated with them, this observation would be tolerably conclusive as to the presence of the same green pigment in *Convoluta Schultzei*. But if Pringsheim's theory that the chlorophyll acts only as a "screen" be true, and if the decomposition is actually effected by the protoplasm of the vegetable cell, then it seems not very unlikely that one green pigment might act as a screen as effectually as another, and the physiological evidence as to the identity of the two pigments would have little value. Some value must, however, be ascribed to it, for it is, on the whole, more probable—even if we accept the screen theory—that only substances belonging to Sorby's groups of chlorophylls,

xanthophylls and lichnoxanthines can effectually act as screens than that other green pigments of a different nature can play that part.

Mr. Geddes has not adduced spectroscopic evidence similar to that given by Sorby in relation to *Spongilla*, in favour of the view that the green pigment of *Convoluta Schultzei* is caused by a mixture of blue and yellow chlorophyll, xanthophyll and lichnoxanthine, or by any one or two of these bodies; nor has the precise structural form in which the pigment occurs in *Convoluta Schultzei* been described, so that we can compare it with the chlorophyll bodies of plants.

Physiological evidence in favour of the assimilation of the green pigment of *Hydra viridis* to that of green plants was obtained two years ago by Mr. J. E. Blomfield, of Magdalen College, Oxford, and University College, London. He has kindly supplied me with the following account of the experiments made by him:

"A number of *Hydra viridis* (some forty or fifty) were placed in a test-tube, and the test-tube filled with water was inverted over water in a basin, the bottom of which was lined with clay so that the test-tube could be pressed into it (*not* so as to completely close the tube) and maintained in an upright position, and the *Hydræ* could either crawl up the tube or remain resting upon the clay. Any gas given off from the *Hydræ* would necessarily ascend in the tube and collect at the top of it, expelling a corresponding volume of water from the inverted mouth of the tube. On exposing several tubes thus prepared to direct sunlight minute bubbles appeared on the *Hydræ* and on the side of the tube, which ascending formed a large bubble at the top of the latter.

"A test-tube containing nothing but water will, when exposed to direct sunlight, be found to have its sides dotted with minute bubbles, which collect into a larger bubble on rising to the top of the column of liquid, but the gas so separated *is reabsorbed when the temperature of the water falls* from the point which it has reached by the heating effect of insolation.

"Several test-tubes containing *Hydræ* were set up in this way and left for a week, but owing to the unfortunately small amount of direct sunlight at the time, only a small bubble of gas was obtained at the top of each tube. The bubbles so obtained were passed from the collecting tubes into a smaller test-tube for analysis. The small test-tube containing some water and the collected gas was inverted over mercury, and a solution of pyrogallie acid in KHO was introduced into the tube. One third of the total volume of

gas was absorbed, and it was accordingly inferred that one third part by volume of the gas evolved from the Hydræ was oxygen gas."

Mr. Blomfield further observed that the green pigment of the Hydræ was soluble in alcohol, and that the green solution obtained in that medium was converted in the course of half an hour's exposure to direct sunlight into a faint yellow colour. Unfortunately nothing further was observed with the spectroscope than the presence of one intense absorption band in the red similar to that afforded by solutions of leaf green.

Morphological evidence with regard to the occurrence of chlorophyll in animals.

#### A. SPONGILLA.

*Chlorophyll-corpuses*.—I now come to the statement of my own observations with reference to the *form* under which the green pigment of *Spongilla* presents itself, and I am of opinion that these observations lead to the conclusion that the green pigment there present, which Sorby has demonstrated to agree in spectroscopic characters of a detailed kind with the green pigment of higher plants, is contained in "chlorophyll-corpuses" or "chlorophyll bodies" which have the same nature as the bodies so designated in the tissues of green plants, and that these corpuses are, as in green plants, formed by the activity of the protoplasm of the cells in which they occur.

In figs. 1 and 2, and others on Plate XX, amœboid cells from green-coloured specimens of *Spongilla fluviatilis* are represented, showing the green-coloured corpuses embedded in their substance, which I consider as chlorophyll-corpuses proper to these cells. The corpuses are concavo-convex discs, averaging  $\frac{1}{10,000}$ th to  $\frac{1}{12,000}$ th inch in diameter. They are of a uniform green colour, and are often so abundant as to occupy a large bulk of the cell. Some cells, however, are observed in which they are much less abundant.

Rarely I have observed in the amœboid cells containing chlorophyll-corpuses of normal size and shape one chlorophyll-corpuscle abnormally large and differing in shape from those usually seen. In fig. 2 *ccc* such a corpuscle of spherical form is drawn. In fig. 12 a similarly large chlorophyll-corpuscle from *Spongilla* is drawn. In this case the green pigment is confined to a superficial layer or shell investing a colourless substance and to a few grains within.

The amœboid cells and the corpuses containing them may be well observed by simply teasing a piece of a living



specimen of green-coloured *Spongilla*. But a valuable method is that of teasing the piece of *Spongilla* in a drop of dilute solution of osmic acid ( $\frac{1}{2}$  per cent.). Such preparations may be subsequently stained by picro-carmin, as shown in Pl. XX, fig. 13. When this treatment is adopted it is noted that though the nucleus of the sponge-cell stains very intensely no staining of the chlorophyll-corpuscle, or of the protoplasm close to it, occurs.

There is no evidence of any nucleus-like body, either within the chlorophyll-corpuscle or in immediate relation with it.

When a piece of green *Spongilla* is decolorised by the action of strong alcohol and subsequently teased the concavo-convex discs, which were before observed in a green state, are still to be found, but now they are colourless.<sup>1</sup>

When the amœboid cells of *Spongilla* containing chlorophyll-corpuscles are broken up on the stage of the microscope the corpuscles are set free, and are found to have a considerable degree of resistance to the action of water and a permanence of form, as is observed with the chlorophyll-corpuscles of plants.

Under such treatment it is further observed that the green concavo-convex discs do not separate cleanly from the protoplasm, but each carries with it a little sphere of protoplasm, upon which it rests as a cap (figs. 9 *d*, 10 *b*). The relation of the green cap to the sphere is such as to suggest a one-sided formation of green matter upon one hemisphere of the protoplasmic particle. Were the formation of green matter symmetrical we should have the protoplasmic sphere enclosed in a complete shell of green substance, as in the abnormal corpuscle of fig. 12, and as in the normal green corpuscles of *Hydra viridis* (figs. 17, 20).

I could not discover in the unbroken amœboid cell that there was any differentiation of a protoplasmic sphere corresponding to each chlorophyll-corpuscle. It would seem rather as though a piece of the surrounding protoplasm simply adheres to the concavo-convex disc when the cell is broken up. At the same time pieces of protoplasm may be observed when such cells have been broken up which connect two or more (as many as six) concavo-convex discs of green colour (fig. 10 *a*), suggesting that the cap-like chlorophyll-corpuscles are grouped around a centre of growth, and

<sup>1</sup> Accordingly we distinguish in the concave chlorophyll-corpuscle of *Spongilla*—(1) the chlorophyll itself, (2) the chromophorous substance which carries the chlorophyll. In *Hydra*, as will be seen subsequently, we have to add to these two elements (3) colourless protoplasm, enclosed by the chromophorous substance.



that they have originated by a process of cleavage of an original layer of green substance which invested the particle of protoplasm. Evidence of the cleaving of the chlorophyll-corpuscles, so as to form two corpuscles from one, is given in the accompanying drawings (fig. 9 c).

I am not acquainted with any chlorophyll bodies of plants which assume the form of concavo-convex discs as do those of *Spongilla*. At the same time there is nothing inconsistent with what is known of chlorophyll bodies in this form, whilst in their simple negative characters the green corpuscles of *Spongilla* are like the chlorophyll bodies of higher plants. They are quite unlike any known forms of unicellular *Algæ*.

*Amyloid substance in Spongilla.*—Neither before treatment with alcohol nor after it did the addition of iodine solution to the sponge-cells reveal any substance *within* the corpuscles, which by its blue or violet coloration could suggest the presence of starch.

I, however, obtained in both green and colourless specimens of *Spongilla* treated in this way with iodine solution abundant evidence of the presence in other regions of the sponge-cell of an amyloid substance. My observations were made on specimens taken late in the year (October), and I am inclined to believe, from my recollection of former experiments, that the amyloid substance is not so abundant in the early part of the year as in autumn.

The amyloid substance occurred in two forms—(1) as a homogeneous substance occupying very large vacuoles—usually only one—in the protoplasm of the sponge-cell (Pl. XX, figs. 3, 4), and (2) as fine spherical granules, which were accumulated on the surface of some of the sponge-cells, and embedded in the superficial layer of the protoplasm (Pl. XX, figs. 4, 5, 8).

The amyloid vacuoles of *Spongilla* and of other sponges were discovered and described by Keller ('Zeitschr. für wiss. Zoologie,' vol. xxx, p. 572). Keller points out that the vacuoles contain a fluid which stains deep blue or violet when iodine solution is added (in my observations I obtained only *violet* staining), and that the substance so coloured is insoluble, either in ordinary or absolute alcohol or in cold water, whilst potash solution decolorises the stained vacuole and causes the cell to swell up.

Keller's observations undoubtedly prove that we have in these vacuoles a starch-like substance in solution, but it is by no means to be concluded that this substance is identical with vegetable starch.

An addition to Keller's observations on these "amyloid deposits" which I have to record is that the vacuole which stains violet with iodine is also deeply stained by a solution of picro-carmin after previous treatment with dilute osmic acid (Pl. XX, fig. 14).

This carmine staining would lead to the inference that an albuminoid as well as an amyloid substance is present in the fluid vacuole.

Keller appears not to have seen the small granules of amyloid substance which I observed in great abundance both in the superficial protoplasm of cells which contained an amyloid vacuole and in those which were devoid of any such vacuole. These granules may have been formed in the protoplasm of the sponge-cells in the same way as the large vacuoles. On the other hand, it seems very possible that they are minute particles resulting from the bursting of a vacuole, and are taken into the substance of the neighbouring sponge-cells either as a normal process of nutrition or accidentally.

I may say that the amyloid vacuoles were exceedingly abundant in specimens of *Spongilla* taken from the Thames near Windsor, in October, and that *they were equally abundant in pale flesh-coloured specimens of Spongilla and in those of a bright green tint.*

It is of importance to notice that neither granules nor vacuoles of amyloid substance appeared to have any relation to the chlorophyll-corpuscles. At the same time, it cannot be denied, that the probability of the endogenous nature of the chlorophyll-corpuscles and of their non-parasitic character, is greatly increased by the demonstration of the fact that the sponge-cell is capable of forming amyloid substance and depositing it in vacuoles in large quantities.

Definite observations, localising the formation of starch-like deposits in the cells of an animal organism, have hitherto been wanting, although there are various indications in the writings of previous observers of starch or starch-like substances having been obtained from animals.

There can be no doubt that a careful investigation by the physiological chemist of the amyloid deposits of *Spongilla*, and of the substances by which they are preceded and accompanied, and of the precise conditions under which they are produced, would be of great value and interest. I am inclined to believe that this abundant formation of amyloid substance—which is in fact most abundant in specimens of *Spongilla* which are actually breaking up and dying down at the in-coming of winter—has possibly a

relation to the formation of the winter "gemmules," and the providing them with a store of food material.

*Angular corpuscles of colourless Spongilla.*—The fact that *Spongilla fluviatilis* occurs almost as frequently in a colourless or rather pale salmon-coloured state as in the green state, is one of very great importance in relation to the nature and history of the chlorophyll-corpuscles found in the latter form. Whenever *Spongilla* grows with deficient access of sunlight it does not develop a green colour, but it appears to be none the less vigorous. I have seen enormous growths (many pounds weight) of colourless *Spongilla* on the lower surface of a barge removed from the river at Oxford. Frequently also in the locks on the Thames, sheet-like growths of *Spongilla* are seen which are only mottled with green, their colour being in other parts light brown.

This fact, at first sight, seems to tell in favour of the theory that the chlorophyll-corpuscles are parasitic organisms, which can only attack and thrive in such growths of *Spongilla* as are exposed to direct sun light.

An examination of the colourless specimens of *Spongilla* with the microscope at once, gives a very different significance to the facts. In the amœboid cells of the colourless *Spongilla* it is true that no green-coloured corpuscles can be found, *but colourless corpuscles are present*, which appear to be the *same bodies as the chlorophyll-corpuscles* in a modified condition. These are angular irregular corpuscles of the same average diameter as the chlorophyll-corpuscles (figs. 6, 11, 14, Pl. XX), and occurring in the same abundance. If cells be taken from a piece of sponge of a *pale* green colour, which is, so to speak, becoming green, individual cases may be observed in which there are one or two chlorophyll-corpuscles present amongst the angular colourless corpuscles. In such specimens too colourless corpuscles may be detected, which assume the concavo-convex shape of the normal chlorophyll-corpuscles (fig. 7).

It is difficult to avoid the conclusion that the colourless angular corpuscles are capable of either directly developing into chlorophyll-corpuscles under the influence of sunlight, or that in the process of their development they can be so modified by the influence of sunlight as to become, instead of angular colourless corpuscles, concavo-convex chlorophyll-corpuscles.

An important fact in this connection, which I think goes far to prove that the chlorophyll-corpuscles of *Spongilla* are formed by the protoplasm of the sponge-cell, was published by



me seven years ago in this Journal (1874, vol. xiv, p. 400). I found that when a piece of colourless *Spongilla* is dipped into sulphuric acid, *it immediately assumes an intense green colour*. It is well known that sulphuric acid has a similar action upon some vegetable cells, which are remarkable for the suppression of what may be considered their normal green pigment. The saprophyte, *Neottia*, is devoid of chlorophyll, but when treated with sulphuric acid certain substances in the protoplasm of its cells appear to develop rapidly a green-coloured body resembling (at any rate in colour) the green pigment of other plants.

The destructive nature of the reagent employed has prevented me from ascertaining, by observing the action under the microscope, whether the green colour thus developed in colourless cells of *Spongilla* arises from a change of the angular corpuscles. It can hardly be doubted that this is the case.

It does not seem possible to hold the view that the colourless angular corpuscles are colourless parasitic Algæ ready to develop into green varieties when exposed to sunlight! They have even less of the form and structure of independent organisms than have the green corpuscles of the verdant varieties of *Spongilla*.

*Dr. Brandt's observations and conclusions with reference to the chlorophyll-corpuscles of Spongilla.*—Dr. Karl Brandt has recently published certain observations with reference to the green-coloured corpuscles of both *Spongilla* and *Hydra*, which lead him to the conclusion that these bodies are not “chlorophyll-corpuscles” similar in nature to the “chlorophyll bodies” of plants, but parasitic or “symbiotic” unicellular Algæ.

It is to be hoped that Dr. Brandt will soon publish his observations more in detail, together with illustrative figures. In the memoir which he has already issued Dr. Brandt makes a series of statements, which are applied by him both to the chlorophyll-corpuscles of *Spongilla* and to those of *Hydra*.

He observes :

(1) That he studied the chlorophyll-corpuscles when isolated from the tissues of the animal by means of pressure.

(2) That the corpuscles thus isolated are not equally and completely green, but possess besides the green-coloured substance always some portion of hyaline protoplasm.

(3) In the hyaline colourless part of the green bodies a *cell nucleus* could in all cases be detected with absolute certainty by treatment with hæmatoxylin. Sometimes more



than one such nucleus was observed (2 to 6), which were regarded as indications of a process of division.

(4) The green corpuscles were maintained on the object slide after isolation from the surrounding cell-protoplasm, and were observed to retain their form for several days or even weeks. When exposed to the light such green corpuscles (from *Spongilla* as well as from *Hydra*) develop starch-grains within their substance.

(5) Isolated chlorophyll-corpuscles from *Spongilla* were brought into association with ciliated Infusoria, which swallowed the chlorophyll-corpuscles. The corpuscles were either digested or ejected unchanged.

On the other hand, the larger chlorophyll-corpuscles of *Hydra viridis*, when similarly swallowed, were found to remain unchanged in the Infusoria for a certain time. Dr. Brandt does not state that these latter corpuscles multiplied by division in the body of the Infusorian which had swallowed them.

Upon the grounds summarised in these five paragraphs, Dr. Brandt concludes that chlorophyll is never formed by animal organisms, but when found in animal cells is due to the presence of parasitic Algæ, to which he has given generic and specific names.

It seems to me that even if we accept every word of Dr. Brandt's statement as to the structure of the chlorophyll-corpuscles there is not sufficient ground for adopting his conclusions. With regard to the statement contained in paragraph 2, I am in agreement with Dr. Brandt.

As to the existence of a cell-nucleus (paragraph 3) in the chlorophyll-corpuscles either of *Spongilla* or *Hydra*, I am at variance with him. I have not used hæmatoxylin as a staining agent in this inquiry, but picro-carmin, and I had very fully satisfied myself that nothing like a cell-nucleus exists in connection with the green corpuscles in either *Spongilla* or *Hydra* previously to Dr. Brandt's statements. I have examined them in various ways, including that of removing the green pigment before treatment with picro-carmin. I have found that when a slight staining only is used, sufficient to colour well the nucleus of the amœboid sponge-cell or of the endoderm-cell of *Hydra*, no coloration of the protoplasm in connection with the chlorophyll-corpuscles is to be seen; but if a strong staining be allowed to take place, then the protoplasm of the general substance of the cell becomes pink as well as the nucleus of the cell, but in a less degree; and if the chlorophyll-corpuscles be squeezed out of cells of *Spongilla* so treated, then the little

piece of protoplasm adherent to each (fig. 9 *d*, Pl. XX) will be seen to have a pink colour, but is still perfectly homogeneous.

Similarly the colourless protoplasm within the green corpuscles of *Hydra* will take up a pink colour when strong staining with picro-carmin is used; but nothing of the nature of a nucleus have I ever seen in these corpuscles, although the little granules within the corpuscles of *Hydra* might lead to the impression that a nucleus is present, (figs. 20, 23) if one were not acquainted with their true nature, as isolated granules of green-coloured substance lying within the corpuscle.

It seems to me *possible* that Dr. Brandt has been misled by these granules. At the same time it is possible that hæmatoxylin brings into view a nucleus-like structure, which picrocarmin does not. Even if this were the case, when we remember that the chlorophyll-bodies of plants are looked upon by botanists as similar in their nature to nuclei, it does not seem that we should have any ground for regarding the chlorophyll-corpuscles as independent organisms.

Dr. Brandt's observation of the formation of starch in the isolated chlorophyll-corpuscles (paragraph 4) is extremely interesting and important. It does not seem to me to tend in any way to prove that the corpuscles are independent organisms. It would simply prove (if fully established) that a bit of protoplasm with its associated envelope or cap of green substance can retain its vital activity just as a piece of an *Amœba* can. At the same time what I note as especially interesting is that Dr. Brandt does *not* state that he has observed starch-grains in association with the chlorophyll-corpuscles when observed in fresh living cells of *Spongilla* (or of *Hydra*). I have failed to detect starch in such position in living *Spongilla*-cells, though I have found abundant amyloid substance in other parts of the sponge-cell. I have also, only in the rarest cases, found a minute trace of starch in association with the chlorophyll-corpuscles of *Hydra viridis*.

This absence of starch from the living chlorophyll-corpuscles when in the sponge-cell, or *Hydra*'s endoderm-cell, must necessarily appear remarkable. I have been driven to the conclusion that the activity of the chlorophyll-corpuscles in these animals in sunlight gives rise to a body similar to that which arises under the same conditions in plants, but *that in place of being deposited in the corpuscle as starch-grains*, it is rapidly diffused and chemically changed

in the surrounding protoplasm of the cell. In *Spongilla*, under certain circumstances, it is deposited as amyloid substance (after diffusion) in the large vacuoles described above and figured in Pl. XX, figs. 1, 3, 4, 14.

Now it appears not improbable that by removing the chlorophyll-corpuscles from the mass of surrounding protoplasm, Dr. Karl Brandt has found a method by which *the product of the activity of the chlorophyll-corpuscle may be, as it were, forced to remain in the corpuscle*, there being no surrounding protoplasm to take it up and operate further upon it. Hence, possibly enough, we get a deposit of starch-grains in the isolated corpuscle which would never occur in the normal condition, since the product of assimilation is in that condition rapidly diffused and so removed from the chlorophyll-corpuscle.

The inquiry suggested by Dr. Brandt's observation on this point seems likely to have valuable results.

With regard to Dr. Brandt's experiments in infecting Infusoria with the supposed parasites of *Spongilla* and *Hydra* (paragraph 5), it is at once apparent from his account of them that they are *opposed to* and not in favour of the parasitic theory.

The chlorophyll-corpuscles of *Spongilla* were digested or else ejected by the infected Infusoria. In other cases the chlorophyll-corpuscles of *Hydra* remained in the Infusorian's body *unchanged*. Had Dr. Brandt's view been confirmed, the green-corpuscle ought to have multiplied in its new host, and even such evidence of a temporary manifestation of vitality after removal from the *Hydra* or *Spongilla* would not in my opinion be at all conclusive to the effect that the chlorophyll-corpuscles are independent organisms, and not parts of the protoplasm of the cell in which they are normally found.

#### HYDRA VIRIDIS.

Professor Nikolas Kleinenberg, in his memorable work on 'Hydra,' has given an account of the chlorophyll-corpuscles of *H. viridis*, which leaves little to be added on the subject. He has not, however, given any of the special features of the chlorophyll-corpuscles in his plates, and the figures which are given in my Pl. XX, figs. 15—27, are, I believe, the first which adequately represent those bodies. Kleinenberg says of them:

"They consist of (1) a dense ground-substance, very rich in albumens, which stains dark brown with iodine, deep red with carmine or aniline; (2) and spread over this an



excessively thin coating of a green colouring matter, which to judge by its chemical and optical characters is either identical with chlorophyll or very near to it. These corpuscles therefore exactly correspond, in regard to their construction, with the chlorophyll-bodies of plants. In a certain number of them the surface is quite smooth, others acquire a segmented appearance from grooves and fissures. (See Pl. XX, fig. 17*a*.) Related to the latter are small corpuscles in the course of breaking down, which exhibit angular forms, and instead of a bright green have a dirty dark colouring (Pl. XX, fig. 21), and gradually pass over into very small dark brown or even black granules, adhering to one another in little heaps (Pl. XX, fig. 19). The number of all these bodies varies greatly according to the nutritional condition of the animal. The green bodies are found chiefly in the marginal region of the cell, and only when they are very abundant, in its basal portion; occasionally they appear to be, as it were, stuck on to the cell-surface, but nevertheless always possess a thin envelope of protoplasm. The free end of the cell never contains chlorophyll-corpuscles; on the other hand, the brown and black granules are accumulated in that region.

“In *H. aurantiaca* and *grisea*<sup>1</sup> the endoderm-cells of the foot and tentacle cavities are devoid of form-elements comparable with the chlorophyll-corpuscles of *H. viridis*. Only orange, brown, and blackish spherical or angular corpuscles occur, which all exhibit a remarkable resistance to chemical reagents. The epithelium of the stomachal cavity contains however—at least in well-nourished specimens—colourless round or oval dense albuminous corpuscles (Pl. XX, fig. 16), which, excepting the absence of chlorophyll, closely resemble the coloured corpuscles of *H. viridis*, and also exhibit the same transitional forms leading to the dark granules.”

I have introduced into this quotation from Kleinenberg references to the figures of the plate accompanying the present memoir. The only important addition which I have to make to Kleinenberg's statement, as to the structure of a normal chlorophyll-corpuscle of *Hydra viridis*, is that very usually there is within the shell or crust of the green-coloured substance one or more minute granules, also coloured green and embedded in the colourless protoplasm of the corpuscle.<sup>2</sup>

<sup>1</sup> Apparently a synonym of our *H. fusca*.

<sup>2</sup> I would, however, analyse the chlorophyll-corpuscle of *Hydra* into three elements as follows—(1) chlorophyll or green pigment which can be dissolved out by alcohol, (2) chromophorous substance, which carries the



Sometimes these are absent, and it seems that specimens of *Hydra viridis* may be obtained, according to season and state of nutrition, in which such internal granules are present in all the corpuscles, or, on the other hand, absent from all or nearly all of them.

The corpuscles average about twice the diameter of the chlorophyll-corpuscles of *Spongilla*, that is to say, from  $\frac{1}{6300}$ th to  $\frac{1}{3300}$ th of an inch. Some are larger. The chief difference between the corpuscles in the two animals is found in the fact that the green substance forms a concavo-convex cap upon its related protoplasmic base or corpuscle in *Spongilla*, whereas in *Hydra* the coloured cap is extended on all sides, so as to form a hollow sphere enclosing some protoplasm, and additional granules are developed within the sphere. It has been pointed out above that, as an exception, a green corpuscle may be found in *Spongilla* having the form characteristic of *Hydra* (see Pl. XX, fig. 12); so, too, in *Hydra* small green corpuscles may be found, which have the green investment incomplete and cap-like (Pl. XX, fig. 20 *a, f, h*). Kleinenberg observed the staining of the colourless protoplasm within the green corpuscles of *Hydra* by iodine, carmine, and aniline. This I have also observed, but found it to be much less intense than that of the cell-nucleus, so that it is possible to obtain a staining of the nucleus whilst the corpuscles remain unstained (Pl. XX, fig. 15).

Kleinenberg makes no mention of any nucleus-like body within the green corpuscles, and I am in accord with him. Dr. Brandt states (*loc. cit.*) that a cell-nucleus can be clearly demonstrated in each chlorophyll-corpuscle, and makes this his chief ground for regarding the corpuscles as parasitic *Algæ*. I have not found such a nucleus in *Hydra* any more than in *Spongilla*, and find it difficult to believe that, if present, it would have escaped the careful examination made both by Kleinenberg and myself.

Kleinenberg makes no mention of starch in connection with the chlorophyll-corpuscles or other parts of the endoderm-cells of *Hydra*. I have also not succeeded in finding starch in these bodies. I have, however, very rarely obtained a blue coloration with iodine in the neighbouring protoplasm, and in one specimen a few granules (not chlo-

chlorophyll, and is usually in the form of a spherical shell, enclosing factor No. 3, but may also occur as granules embedded in that third factor; the chromophorous substance *resists* the action of staining agents. (3) Colourless homogeneous protoplasm, enclosed by the shell of chromophorous substance, and capable of taking a stain with strong colouring agents, but devoid of nucleus or nuclear matter.

rophyllaceous), previously colourless, lying in the cell-protoplasm, gave a blue colour with iodine. I would venture the surmise that in *Hydra viridis*, as in *Spongilla*, the product of assimilation due to the activity of the chlorophyll-corpuscles is very rapidly diffused, and does not take the form of starch as in green plants, or if it does take that form, it is after diffusion from the seat of assimilation.

A very strong argument against Brandt's theory of the parasitic nature of the chlorophyll-corpuscles is found in the fact noticed by Kleinenberg, that minute angular fragments of a green colour are often present, together with the normal corpuscles (Pl. XX, fig. 21). In fact, in *Hydra*, as in *Spongilla*, though there is a *normal* and fairly constant form of chlorophyll-corpuscle, yet other irregular forms appear side by side with these. How can such irregular forms be explained on the parasite theory? They present no difficulty if the corpuscles are regarded as products of the animal's cell-protoplasm, for it may well be that such products should sometimes be incompletely formed or of monstrous size and shape.

The brown and blackish granules noted by Kleinenberg as occurring both in *H. viridis* and in the "greenless" varieties of *Hydra* are important (Pl. XX, fig. 19). They appear to result from the breaking down of the chlorophyll bodies or their colourless representatives.

Professor Jeffrey Parker, in his paper on the endoderm of *Hydra* ('Quart. Journ. Micr. Sci.,' vol. XX), has advanced the view that these dark granular bodies are ingested food particles in the course of digestion. In this view I cannot agree. They appear to me to be undoubtedly in the case of *H. viridis* connected with a degeneration of a chlorophyll-corpuscle, and in *H. fusca* I am inclined equally to attribute them to the formative activity of the cell-protoplasm. Similar dark-coloured granules in the endoderm of *Cordylophora* and other hydroids (probably also the very scarce black granules in the endoderm of the *Medusa Linnocodium*, 'Quart. Journ. Micr. Sci.' (vol. XXI, Pl. VIII, fig. 1 *b*), are also to be regarded as products formed by the cell, and not as ingested particles. However, it is difficult to distinguish one kind of "dark granule" from another, and it is possible that *some* such dark granules, observed in the endoderm-cells of *Hydrozoa*, are really food particles which are undergoing intra-cellular digestion.

*The representatives of chlorophyll-corpuscles in colourless and olive green Hydræ.*—It is an open question as to whether the white, brown, and orange-coloured specimens of

*Hydra* are to be regarded as species distinct from *Hydra viridis* or as varieties. I incline to take the latter view, since transitional forms are met with, namely, olive-green and bluish-green specimens, which have incompletely developed chlorophyll.

There is no such clear evidence of the specific identity of *Hydra viridis* and *Hydra fusca* as there is of the identity of colourless and green varieties of *Spongilla*. In the latter case one and the same piece of sponge may be found green where exposed to sunlight, and pale flesh colour where shaded from the light.

The experiment has not, I believe, been made of maintaining *Hydra viridis* in obscurity, though Max Schultze obtained in this way colourless individuals of *Vortex viridis*.

Commonly in this country large numbers of a very pale brown *Hydra* are found associated without the presence of any green specimens. These colourless *Hydræ* (*H. fusca*) are larger than the *H. viridis* usually is. But I know of no character separating the two beyond colour and size. The larger size of *H. fusca*, if it be regarded as a variety of *H. viridis*, may well be correlated with the non-development of chlorophyll, and a less active growth. For though the individual is large it may possibly be less active in budding in *H. fusca* than in the smaller *H. viridis*. Small size and rapid fission may go well together with the nutritional advantages represented by the possession of chlorophyll-corpuscles.

In the endoderm-cells of such specimens of *H. fusca* (as described by Kleinenberg for his *H. aurantiaca* and *H. grisea*) there are angular and rounded colourless bodies (Pl. XX, fig. 16 g), which appear to represent in a colourless state the green corpuscles of *H. viridis*. At the same time they are not definitely spherical, as are the latter. Just the same kind of difference is observed in relation to these corpuscles between *H. viridis* and *H. fusca* as there is between green and colourless *Spongilla* in relation to their corpuscles.

I have not made the experiment of subjecting *Hydra fusca* to the action of sulphuric acid, a reagent which, as narrated above, develops a green colour in colourless samples of *Spongilla*. But I have examined carefully some very interesting specimens of *Hydra* which were found by my assistant Mr. A. G. Bourne in company with *Hydra fusca*, but which were of an olive green or dull blue green instead of pale brown.

These exceptional individuals appear to me to have great interest. I found in their endoderm-cells that a certain amount of green pigment was developed, but instead of the



green bodies having the form of spherical corpuscles, they formed irregular angular masses, as shown in Pl. XX, fig. 18. Also, I was able to obtain, by squeezing from one and the same endoderm-cell, groups of angular granules arranged symmetrically as parts of a sphere, of which *some* were colourless, whilst others were green (Pl. XX, fig. 22).

I consider this strong evidence in favour of the view that the colourless angular bodies of *H. fusca* are potentially chlorophyll-corpuscles; that is to say, under certain circumstances they may develop in themselves chlorophyll. What the conditions are precisely, we are as yet unable to say.

It is noteworthy that both in *Spongilla* and in *Hydra*, when the pigment bodies remain in an abortive condition, they are irregular and angular; when they develop chlorophyll green on the other hand by peripheral activity, they tend to the spherical condition. This may, it seems, be connected with the fact that the formation of the chlorophyll is essentially a *surface* activity probably dependent on the access of sunlight, and this surface activity would, if perfectly symmetrical, necessarily result in the production of a sphere.

*Dr. Brandt's conclusions with regard to the chlorophyll-corpuscles of Hydra viridis.*—The summary given a few pages back of Dr. Brandt's statements in reference to the corpuscles of *Spongilla* applies equally to those of *Hydra*.

I am unable to see that he has adduced any facts, excepting the presence of a nucleus, which I doubt, which tend to the conclusion which he has so definitely formulated by assigning to the green corpuscles of *Hydra* the name *Zoochlorella conductrix*.

There is in all that is known of the structure of the chlorophyll-corpuscles of *Hydra*, as of *Spongilla*, nothing which separates them in character from the known chlorophyll bodies of plants. On the other hand, it is not only impossible to characterise Dr. Brandt's genus "*Zoochlorella*" in botanical language, but there are a variety of facts known both as to the objects called by him *Zoochlorella conductrix* and as to those called *Zoochlorella parasitica*, which lead to the conclusion that in thus dogmatically asserting the parasitic and algoid nature of those objects, Dr. Karl Brandt has wandered very far from the legitimate inferences warranted by the facts.

No distinct wall, either of cellulose or of other substance, exists external to the green-coloured cap or shell of the chlorophyll-corpuscles of *Hydra* and *Spongilla*. Their



form, especially in the latter, is very varied. In some the green colour is very partially deposited in granules and superficial caps, *in others it is absent altogether*, and the corpuscle is irregular and angular in form.

Conclusion as to the parasitic or non-parasitic nature of the chlorophyll-corpuscles of *Hydra* and *Spongilla*.—The final conclusion to which we are led in relation to the chlorophyll-corpuscles of *Spongilla* and *Hydra* is that a careful study of these bodies reveals in both cases their correspondence with the known structure of the chlorophyll bodies of plants, and that those who, like Semper and Brandt, have supposed these chlorophyll-corpuscles to be parasitic, have been misled by, firstly, an imperfect acquaintance with the characters of chlorophyll bodies in general and of these in particular, and, secondly, by the plausible but delusive analogy presented by the “yellow cells” of Radiolarians and of Anthozoa.

As to the nature of these latter bodies, I have no observations to offer.

The chlorophyll-corpuscles of *Spongilla* and *Hydra* in relation to Pringsheim's theory of chlorophyll.—Now that we have established the occurrence of “chlorophyll,” or the combined substances which together constitute that pigment, in *Spongilla*, and with nearly equal certainty in *Hydra*, and also have come to the conclusion that the “chlorophyll” is formed in corpuscles in the cells of those animals just as it is in green plants, it becomes very important to know whether the chlorophyll in the animal is serving the same purpose as it is in the plant; and if so, whether we may not be able to get indications from the animals as to the disputed function of the green pigment, such as plants are unable to furnish.

There is no doubt a field for experimental inquiry here, and with the memoir of Pringsheim in his hand the zoologist may carry out a variety of inquiries upon “animal chlorophyll.”

I would here only briefly insist on one or two remarkable facts which are apparent, and which bear upon the general question of the function of chlorophyll.

In the first place, what we may call “greenless” *Spongilla* and “greenless” *Hydra* flourish abundantly in the same waters with green-coloured *Spongillæ* and green-coloured *Hydræ*. Hence, whatever value attaches to the chlorophyll—it cannot be a very great one in relation to the vital processes of these animals.

In the second place, no starch can be found in immediate

relation to the chlorophyll-corpuscles of either green Spongilla or green Hydra, when in normal conditions. But, on the other hand, an amyloid substance is formed by the green sponge-cell, and stored up in large quantities in vacuoles.

*Equally large* quantities of starchy matter are formed by greenless Spongilla. Accordingly, the Spongilla is not *dependent* upon chlorophyll for its power of forming amyloid substance. This formation of amyloid substance *appears* to be due to a synthetical process resident in the colourless protoplasm of the sponge-cell. It is not yet *known* that the process is a synthesis, or that the decomposition of  $\text{CO}_2$  is connected with it; but if this could be proved to be the case, we should have strong evidence in favour of the "screen theory" of chlorophyll. For we should then have the amyloid synthesis going on equally both in green and "greenless" Spongilla, in the former the protoplasm being protected by chlorophyll from the direct sunlight, in the latter no such protector being required nor developed, and this because the greenless Spongilla exists in deep shade away from the reach of those rays which it is the business of chlorophyll to intercept.

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## MEMOIRS.

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NOTE *on the* FORMATION *of* FIBRINE. By MRS. ERNEST HART.

(With Plate XXI.)

THE research, of which this paper is a record, was made in the laboratory of M. Ranvier, at the Collège de France, in the Autumn of 1879. It has remained so long unpublished because I was always in hopes of being able to make the work more complete and exhaustive. In presenting it now for publication and criticism, I do so with great diffidence, knowing that the work is incomplete; yet I am assured by competent authorities that, viewed in connection with other work on this subject now being carried on in various parts of the world, it may not be without utility and suggestiveness, as an addition to the data for the investigation of the production of fibrine.

At the conclusion of the little paper which I published in the 'London Medical Record' in January, 1880, on the Norris corpuscle, I stated my opinion that the colourless corpuscles described by Dr. Norris, and seen by him and others who follow his methods, "are red corpuscles that have undergone post mortem changes prior to taking part in the formation of the fibrine. On this subject I hope shortly to publish some further observations." It was in repeating Dr. Norris's work on the invisible corpuscles, and by means of the very ingenious methods which he has invented to obtain exceedingly fine films of blood, that I observed the appearances I am about to describe. It will perhaps be remembered that Dr. Norris's methods consist in what he calls "isolation" and "packing." The method of "isolation," which is that which I found so very useful, is as follows:—A perfectly smooth and level slide is chosen, and at some slight distance from the centre a small hole is drilled into which a metal eyelet is inserted, care being taken that the metal edge is not raised above the glass surface. A smooth and level thin cover-glass is now chosen and strapped on to the slide by means of a narrow piece of diachylon plaster, so that the free

edge exactly overlaps the metal eyelet; a screw fitting the eyelet is then inserted into the hole, and the method of procedure is as follows:—The tip of the finger is pricked and a drop of blood is placed, as rapidly as possible, at one of the free edges of the cover glass; the blood enters by capillary attraction, forming a delicate even layer between the two glass surfaces; the slide is then inverted over a shallow vessel containing a 2-per cent. solution of osmic acid, and the hinged cover glass is gently raised by passing the screw further through the eyelet. All the fluid particles, and the great majority of the corpuscles, flow immediately towards the hinged edge of the cover glass, leaving only a few red corpuscles, and here and there a white one, adhering to the glass surface; these are *instantaneously* fixed by the action of the osmic acid vapour—a fact originally pointed out by Professor Ray Lankester many years ago—indeed so perfect and complete is the fixing of the corpuscles on the glass by the action of the osmic acid vapour, that the glass may be immersed for a long time in water, and may even be dried roughly with a towel without displacing or injuring them. Among the corpuscles which have adhered to the glass surface, Dr. Norris discovers, by various means of staining, his invisible corpuscle. That it is there I do not deny, but that it is there because it previously existed in this condition in the blood in the living state is I think open to dispute; in fact, as I stated in my former paper, these colourless discs are in my opinion unstable red corpuscles, or corpuscles of low resistance, which have parted with their hæmoglobin, possibly simply by the fact of the withdrawal of the serum.

In continuing these investigations and in repeating this experiment of “isolation” a great number of times, I began to observe that the appearances changed according to the length of time which elapsed between the spreading of the layer of blood between the two glass surfaces and the moment when the cover glass was raised; and thus discovered that a whole series of phenomena could be traced, leading from the pale or colourless corpuscle up to the complete formation of networks or bands of fibrine. In developing this method of working I found that the staining reagents recommended by Dr. Norris were not sufficiently powerful to bring out all the details that could be observed on the glass surfaces, and after many trials I found that a highly concentrated solution of nitrate of rosanilin in absolute alcohol was the best staining reagent to use. The method I adopted was to detach the cover glass from the slide after the corpuscles had been fixed by the osmic acid vapour, and to examine both the surfaces of the cover glass and the slide under the microscope, to see which presented the most perfect



preparations. Having made a selection I deposited a drop of the concentrated solution of nitrate of rosanilin on to glass and allowed it to remain for a few moments, then washed it off with a fine jet of distilled water. The red, pale and colourless corpuscles, with their ramifications and the most delicate fibrils of fibrine, then become visible under a high power. These preparations may be mounted dry and will keep for a great length of time. If the process be performed as rapidly as the dexterity gained by an oft repeated experiment will allow, the appearances presented in fig. 1 will be seen. In this it will be observed that the circular appearance of the corpuscles is perfectly preserved, and that every shade of colour may be found, from the normal red corpuscles down to the colourless Norris corpuscle, which only takes the faintest tint of pink. If, however, the glass surfaces be allowed to remain in contact for a moment, the colourless corpuscles are found to have lost their globular form and to have become pyriform or elongated, as shown in fig. 2. On leaving the glass surfaces still longer in contact, these pale corpuscles are observed to undergo a remarkable change, they send out long processes or tails, which bifurcate and divaricate in every direction. Fig. 3 gives some specimens of these branching cells carefully drawn with the camera lucida; they were, it is true, not all obtained from the same plate, but have been grouped together for convenience. Fig. 4 gives perhaps a more remarkable specimen of these branching corpuscles. All the former specimens are from human blood, but figs. 4 and 5 are from rabbit's blood. On allowing a still longer interval to elapse, so that it is more than probable that coagulation would occur in a film of blood lying between two glass surfaces, and on separating these surfaces, perfect specimens of fibrine may be obtained after staining. These are represented in figs. 6 and 7. On now searching the field the pale corpuscles, which could formerly almost always be discovered, are nowhere to be found, and the conclusion is forced upon one that the branching corpuscles have developed or broken down into fibrinous threads. Small granules (D., fig. 6) are, however, found from which threads of fibrine appear to spring. These granules are described in M. Ranvier's "*Traité Technique d'Histologie*," as the centres of fibrine formation. They appear to me to be all that is left of the pale corpuscles, whose intermediate transformations have not before been recognised, but may, I believe, perhaps be identified with the appearances and changes which I have described and figured. I repeated these experiments a great number of times, with the object of arriving at the exact moment when I should be able to verify this hypothesis, and trace the fibres of fibrine into one or

more of the pale corpuscles, and in fig. 8, which is drawn faithfully from the preparation, this departure of the fibrils of fibrine from the pale corpuscles is, I think, demonstrated.

In the well-known and generally accepted theory of A. Schmidt as to the formation of fibrine, it is assumed that the red corpuscles part with fibrino-plastin before the formation of fibrine can be accomplished. In the course of my research this theory seemed to me to be often capable of physical demonstration by the appearances the red corpuscles sometimes present, for they occasionally appear to be in the act of discharging part of their contents. This appearance is shown in fig. 9, E. The crescentic corpuscles, which are also figured (D., fig. 9), seem to show a loss of substance. They were very frequently found both in human and in rabbit's blood. Of these crescentic corpuscles I am unable to give any satisfactory explanation.

It may be objected that the tails of the colourless and pale corpuscles are produced by currents of the serum. This objection was present to my mind the whole time that these experiments were being performed, and great care was taken to ascertain if the processes pointed in the direction taken by the fluid. In fig. 5 it will be noted, however, that the tails of the corpuscles point in opposite directions, and their position does not seem to me at all to justify the hypothesis that they may be produced by eddies; also it will be observed that in fig. 5 both ends of the corpuscles are sending out processes. In fig. 3, moreover, it may be seen that the processes do not take the direction of any possible current, but that they regularly divide and ramify like the branches of a tree. In fig. 10 two pale corpuscles (C) also will be seen sending out branches, which cross and lie over one another; this one fact militates strongly against the explanation by currents.

Again, it may be objected that the processes sent out by the corpuscles and the bands and fibres represented in figs. 6 and 7 are not true fibrine. This is, I allow, a weighty objection.

As the preparations are all fixed by osmic acid vapour they are, therefore, incapable of being tested by chemical reagents for fibrine. To eliminate this error I may, however, state that I defibrinated fresh rabbit's blood and treated the defibrinated blood in the way already described, with the result of finding many pale corpuscles mixed with the red, (the pale corpuscle being, I imagine, the very first step in the formation of fibrine), but extremely few transparent corpuscles, a few crescents, and no fibrils. I also treated the serum of blood-clot in the same way, and found neither transparent corpuscles nor fibrils. To be certain, also, that the transparent branching corpuscles were not accidental productions, minute

drops of serum coagulated on the glass by the action of the osmic acid vapour, I repeated these experiments with pure white of egg, mixed with distilled water, till it had the consistency of blood, and also with fresh peritoneal and pericardial fluid, and failed to obtain any appearances resembling the pale or the branching corpuscles.

These preparations have been, during the last two years, inspected by many critical histologists and physiologists, who have expressed favorable opinions of the interest of the results, incomplete as they are. I have, however, long entertained the hope that I might have been able before this to have carried the research further, and to have demonstrated the same appearances in the blood by other methods, and under conditions which would have decided beyond a doubt whether, as I at present venture to opine, these branching corpuscles are the fibrine factors of the blood or not. If my opinions are correct and my work is confirmed, it is unnecessary to indicate the great interest which would attach to such an explanation of the formation of fibrine, and the light it would throw on much which has hitherto been obscure and doubtful in the phenomenon of coagulation, both within the body, and in blood withdrawn from the body. Other engagements have prevented me from carrying on the inquiry, and I do not see any immediate prospect of being able to resume it. In publishing this note now, I am sensible of the incompleteness of the observations, but I am led to believe that they may be useful in the present state of this subject, as furnishing material for suggestion and for further inquiry. The drawings were all made by my own hand by the camera lucida, and with the utmost desire to ensure fidelity of outline and correctness of colour, my object being to elucidate facts, not to enforce a theory.

*Postscript.*—This paper was set in type at the beginning of the year, and was intended for publication in the last number of this Journal, but owing to pressure on space it was unavoidably delayed; meantime, Bizzozero, and subsequently Nariis, having made some publications on the subject, some few copies of this paper have, with the editor's consent, been privately circulated.

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*The GENESIS of the EGG in TRITON.* (With Plates XXII, XXIII and XXIV.) By Mr. T. IWAKAWA.

CONTENTS.—Some general remarks, 260. The manner of depositing the egg, 262. The structure of the ovary, 262. Origin of the ovum, 264. Epithelial islands, 266. Formation of yolk-spherules, 271. Vitelline membrane, 273. The germinal vesicle, 274. The "yolk-nucleus," 276. Bibliography, 277. Explanation of plates.

IN the spring of 1880 I began, at the suggestion of my highly-esteemed teacher, Mr. Whitman, to study the early stages in the development of our common Triton (*T. pyrrhogaster*, Boje) (1).

It was my intention, circumstances favouring, to study the whole series of events, from the original egg-cell to the transformation of the embryo. The spring and summer were mainly devoted to a study of the cleavage and the external changes of the embryo. The appearance of the two important memoirs of Bambeke (2) and Clarke (3), covering the same ground, makes it unnecessary to publish my earlier observations.

The present paper, which treats mainly of the genesis of the egg, was begun in the autumn of 1880, and concluded in June, 1881. Owing to the want of proper aquaria I have obtained very few fresh-laid eggs, and have, therefore, failed to make anything more than fragmentary observations on the changes that follow impregnation.

With reference to method of hardening, I have been most successful with the picro-sulphuric acid of Kleinenburg. For staining fluids I have made use of picro-carmin, hæmatoxylin, and Beal's carmin. Silver nitrate was employed to obtain surface views of the ovary.

*Some General Remarks.*

*Triton pyrrhogaster* is very common in this island, being found in ponds, brooks, ditches, and rice fields. It occurs very abundantly in the pond of Inokashira, located about ten miles west of Tokio, from which place most of my speci-



mens have been obtained. The average length of the adult male is 9.5 cm., female 11.7 cm.

The females are generally larger as well as longer than the males. The only external sexual differences, aside from size, are found in the form and length of the tail, and in the size and form of the cloacal lips. In the male the tail is short and broad with an obtuse apex, while in the female it is elongated (and narrow, and somewhat pointed at the extremity. The cloacal orifice of the female is an elongated slit, the lips of which have a swollen appearance. In the male the lips are much larger and the slit much longer, the whole being well adapted for clasping the cloacal slit of the female in the act of copulation.

For keeping specimens I have been obliged to make use of small glass aquaria, the water being kept in a tolerably good condition by such water plants as *Chara* and *Vallisneria*.

Their food consists of worms, insects' larvæ, small molluscs, &c. They will eat pieces of fish, and even grains of boiled rice. When they are extremely hungry they sometimes bite one another. I observed one instance in which a poor small fellow was swallowed up to the middle part of the body by a larger and stronger one; but the latter had undertaken too much, for he was unable to complete the act of swallowing or to disgorge, and finally died, after vain attempts to free himself from the obstinate morsel. I have also observed that among young specimens reared in an aquarium the larger ones sometimes devour the smaller and weaker ones.

Although I have never seen the act of copulation, I think there can be no doubt that such an act takes place, as I have often found spermatozoa in the oviducts.<sup>1</sup> The manner in which fecundation takes place was made known in 1864 by Professor Nauck, in the 'Correspondenzblatt des Naturforscher-Vereins zu Riga.' This paper is known to me only through an article by Dr. J. V. Bedriaga in the 'Zoologischer Anzeiger,' No. 79, 1881, p. 157. As here quoted, Professor Nauck's statements run thus:

"Prof. Dr. Nauck (heisstes auf p. 85 des correspondenz blattes zu Riga), berichtete die von ihm einmal beobachtete Begattung zweier Tritonen. Nachdem bei dem sonst Kammlosen Männchen sich ein Kamm über Rücken und

<sup>1</sup> Professor Gasco's recent observations ('Zool. Anz.,' Nos. 85 and 86) on Triton and Axolotl show that no real copulation takes place in these Urodela, since the spermatid fluid is not conveyed to the female by cloacal contact.

Schwanz gebildet hatte und auch das Weibchen eine stärkere Kammauschwellung zeigte, schwammen beide neben einander her, jedoch so, dass ihre Köpfe entgegengesetzte Richtung hatten. Die Schwänze beider waren im Halbkreise gebogen und berührten sich mit den Spitzen, so dass das Paar die gestalt eines S. darbot.

“Während die so verbundenen Schwänze lebhaft hin und her virbrirten, sah man die Kloake des Weibchens dentliche Schluckbemegungen machen. Durch die Vibration gelangte der männliche Samen an die Kloake des Weibchens und wurde von dieser aufgenommen.

“Die Tritonen legen also nicht, wie viele Amphibien, unbefruchtete Eeier.”

### *The manner of Depositing the Egg.*

Only one egg is laid at one time—precisely how often I have not ascertained. The eggs of those specimens kept under observation were generally deposited during the night or early morning on water plants. I have sometimes found eggs on dead leaves at the bottom of ponds, ditches, &c.

During the act the female generally turns upside down, seizes the plant by the hind feet, gathering the leaves around the cloacal orifice, which is protruded and brought into contact with the plant. In this position the egg is ejected and fixed on the plant by means of the outer gelatinous envelope, which is quite sticky when it first comes in contact with the water.

The entire act lasts about five minutes. The purpose of turning upside down seems to be to place the egg in a concealed position under the leaf or stem.

### *The Structure of the Ovary.*

The ovaries of Triton are a pair of elongated closed sacs, tapering slightly towards either end, the hind ends, in our species, bending forwards. From the inner surface of the thin wall project eggs in various stages of growth.

For obtaining surface views—which have been of more use than sections in determining the origin of the ova—I have employed silver nitrate and mounted in glycerine.

Before mounting, all the larger opaque eggs were carefully removed by the aid of needles, leaving only the thin wall with small and transparent ova.

Silver nitrate, while it browns the protoplasm of the epithelial cells and blackens their boundaries, leaves the nuclei for a time transparent, and inclining to a milky whiteness.

The wall of the ovary (fig. 1) consists of three layers—an external *germinal epithelium* (*g. ep.*), a *lining epithelium* (*l. ep.*), and a middle layer of connective tissue or *stroma* (*st.*).

The cells of the *germinal epithelium*, seen from the surface with a magnifying power of 400 to 500 diameters, generally show smooth hexagonal boundaries, with nuclei (*n.*) lying near the centre, or, more commonly, towards one side of the cell.

The nuclei have a somewhat elongated oval form, the shorter axis measuring about  $\cdot 016$  mm., the longer about  $\cdot 023$  mm.

By lowering the focus the nuclei of the stroma (*st. n.*) are brought into view, together with fine blood-vessels, which traverse the stroma. In this layer no cell boundaries can be recognised. The nuclei are easily distinguished from those of the superficial epithelium, being smaller ( $\cdot 010$  by  $\cdot 013$  mm.), sharper in outline, and elliptical in shape.

Placing the focus still lower, the elongated polygonal cells of the lining epithelium (*l. ep.*) are seen. These cells are bounded by fine wavy lines; the nuclei (*l. n.*) have an elongated oval or elliptical form, and are a little larger than those of the germinal epithelium, measuring  $\cdot 017$  by  $\cdot 024$  mm.

The size of the cells of the germinal epithelium varies from place to place, being much smaller in those areas where primordial ova are forming than elsewhere. This fact may be taken as an evidence that the epithelial cells multiply by division, and that this multiplication is more or less restricted to scattered patches or areas.

Waldeyer (4) speaks of "cell-islands," or scattered aggregations of pavement epithelial cells. In most cases he found these cell-clusters lying beneath the germinal epithelium; but in a few cases (see his fig. 28) some of these cells were found to have a superficial position, the germinal epithelium (endothelium) being absent in such spots.<sup>1</sup> I have made a great many surface preparations, but I have never been able to find any places where the continuity of the germinal epithelium was broken.

If my view of the origin of the ova be correct, it would be difficult to account for such a break in the outer layer as Waldeyer has described and figured, except by supposing that the preparation itself was faulty. Waldeyer's figure

<sup>1</sup> Waldeyer's opinion that the outer layer of the wall of the ovary is not *epithelium*, but *peritoneal endothelium*, rest upon a distinction that finds no support in the latest investigations on the origin of the peritoneum.

which, as he acknowledges, represents a rare instance, would seem to favour his theory of the origin of the ova, according to which *entire* epithelial cells, in larger or smaller numbers, sink into the stroma, and are subsequently overgrown by the closing up of the epithelium. I have done my best to find evidence to support this view, but my observations have led me to a somewhat different conclusion, as will be seen from the following pages.

### *Origin of the Ovum.*

Before stating my own conclusions, I will give briefly those which have been put forward by other authors.

Van Beneden (5), from an extended and comprehensive study of oögenesis, chiefly in invertebrate animals, came to the conclusion that the ovum invariably arises from a *nucleated mass of protoplasm*.

Waldeyer (4), starting from the other end of the animal kingdom—the vertebrates, arrived at the conclusion that the ovum of every egg-producing animal is at first an *epithelial cell*. So far as the vertebrate animals are concerned, this opinion has met with general acceptance.

For Ludwig (6) the chief question is, Does the ovum represent a single cell? His view may be stated in his own words (6, p. 194):

“Our investigations have taught us that in all animals, without a single well-established exception, the egg is a single simple cell from the outset, and that it does not lose this character before maturity.”

Götte (7), on the other hand, has maintained that the primordial ovum of *Bombinator igneus* does not represent a single epithelial cell, but that several such cells coalesce, and thus give rise to a mass of protoplasm, in which are suspended the nuclei of the original cells. Later these nuclei also coalesce, forming a single body. This uni-nuclear mass does not, according to Götte's interpretation, represent a cell, but an unorganised body, which at this stage contains no “proper egg material,” *i. e.* contains no yolk-substance, which alone is the foundation (“Erzeuger der späteren Entwicklung zum selbstständigen Leben!”) of all vital development.

The recent investigations of Dr. Nussbaum (8) have placed this matter in a new light, and have opened up questions of great interest, which can only be decided by embryological studies.

Dr. Nussbaum finds the functional part of the genital organs of *Rana fusca*, in a larva which has attained the



length of 1.4 cm., to consist of a string-like aggregation of cells ("Geschlechtszellen") lying on the median side of the Wolffian duct, in the middle third of the body-cavity. These sexual cells are at this time easily distinguished from neighbouring cells by the presence of yolk elements, which have elsewhere dissolved. They are invested with peritoneal epithelium, which is entirely distinct and independent of them.

They begin now to multiply by division, and each cell resulting from this division becomes separately invested in a capsule of peritoneal cells. Meanwhile the yolk elements have disappeared. About the time the hind legs bud forth, the cells thus enclosed split up each into several cells, and the nucleus in each of these several cells divides, producing a small heap of nuclei. We thus arrive at a stage in which a peritoneal capsule encloses several multinuclear cells. A single nucleus in each of the sister cells enclosed in one capsule now grows larger, while the others in the same cell remain small, and take a peripheral position. The larger nucleus with the protoplasm collected around it forms the primordial ovum, while the smaller peripherally placed nuclei, each invested with a share of the protoplasm of the original cell, become the follicular epithelium.

We have thus a peritoneal capsule enclosing several ova, each of which is surrounded by its own follicular epithelium. The concluding step in the formation of the young follicle is brought about by ingrowths from the peritoneal envelope, which form a second envelope around each ovum. Each ovum has now a *membrana granulosa*, having the same origin as itself, and a *tunica propria folliculi*, which consists of connective tissue derived from the peritoneal envelope of the ovary.

Nussbaum asserts that the same course of development is found in the adult as in the embryo. He thus denies any genetic relation between the peritoneal epithelium and the primordial ova, claiming that both ova and follicular epithelium are derived from "sexual cells," which are from beginning to end distinct from the peritoneal layer.

Dr. Valaoritis (9) has published a preliminary paper, in which he has put forward a new theory of the origin of the egg. He maintains that the ovum is at first a *white blood-corpuscle* ("Leucocyte"), which becomes lodged in the ovarian epithelium, and here matures.

In this paper he appears to base his conclusion concerning the origin of the egg chiefly on the amœba-like power of movement known to belong to white blood-corpuscles, and

assumed to belong also to all young egg-cells. Whatever evidences may be produced in the future in favour of this view, it must for the present be regarded, I think, as a theory without any proper basis.

Automatic action is a character belonging to the protoplasm of young cells in general; and I fail to see any evidence for the assumption that young epithelial cells "execute movements under no conditions."

### *Epithelial Islands.*

The epithelial islands discovered by Waldeyer have been mentioned by other authors, and interpreted in various ways.

Spengel (10), describing the ovary of the Urodela, says, "Its outer surface is covered with peritoneal epithelium, which in the adult animal preserves, *in places*, the character of germinal epithelium, and serves to replace the eggs that have matured."

Brandt (11) succeeded in finding in the frog only a single case comparable with the cell islands of Waldeyer.

Kolessnikow (12) has described "*Keim-epithelinseln*" found in Triton, Salamander, and Frog. Speaking of the frog, he says:—"Groups of peculiar cells are to be seen from place to place between the endothelial cells; these cells show a granular protoplasm, and larger or smaller nuclei; they have sometimes a round, sometimes a polygonal form, and among them lie single cells, distinguished from them in size, size of nucleus, and richness in protoplasm. The contours of these peculiar cells and the bounding lines separating them from the neighbouring endothelial cells are always plain to be seen. . . . I regard the groups of cells just mentioned as islands of germinal epithelium, in which the enlarged cells appear as primordial eggs. The size of the islands is  $\cdot 093$  to  $\cdot 186$  mm., that of the cells  $\cdot 0139$  to  $\cdot 0232$  mm.

"*In places these epithelial cells* are seen to lie under the endothelium, *i.e.* covered by it. In such places they form sometimes deep and broad, sometimes small groups, which often stand in connection with the superficial germinal epithelium."

In regard to the origin of these islands, Kolessnikow expresses himself thus:

"With the transformation of the sexual gland into an ovary begins a more rapid growth of the stroma on the one hand, and a relatively slower growth of germinal epithelium on the other, and the result (of this unequal growth) is that

the germinal epithelium is distributed in small islands, and the stroma cells come to the surface of the ovary, where they take the form of endothelium. The primordial eggs then arise by a (further) growth of single cells of the germinal epithelium, while the enlargement of the ovary results from the growth of the stroma."

Valaoritis (l. c., p. 598) states that these islands are wanting in immature specimens of *S. maculata*, and that the period of sexual activity is distinguished from that of inactivity only by the presence of such islands.

Hoffman (according to Valaoritis) says that "the peritoneal epithelium, which covers the ovary of the land Salamander, is interrupted in places, in which places there is an ovarian epithelium, which serves to replace the eggs that have been used."

From these citations it is apparent that great differences of opinion exist with reference, not only to the precise origin of the primordial ova, but also to the character of the external cell-layer of the ovary.

In regard to the origin of the ovum, we have at least three quite different theories. While differing in regard to the *mode* of origin, the majority of authorities maintain that the ova are derived from the outer cell-layer of the ovary. On the other hand, Nussbaum holds that they arise entirely independently of this outer layer—from so-called "Geschlechtszellen."

Valaoritis agrees with Nussbaum in denying the epithelial origin of the ovum, but claims that it is nothing but a white blood-corpuscle.

The difference of opinion in regard to the character of the outer cell-layer of the ovary has led to confusion in the names applied to it. Some call it "peritoneal epithelium;" some "endothelium;" while others hold that it is made up of two distinct elements, which they distinguish as "epithelium" and "endothelium." In this paper it will be spoken of as "germinal epithelium." I began the investigation of this point in company with a fellow-student, Mr. Sasaki, to whom I am indebted for a few figures, which he has generously allowed me to use. We have made together a very large number of silver nitrate preparations for surface views, extending through the winter and spring months; and also many sections. Our experience has taught us, what others have learned before us, how difficult a matter it is to determine the origin of the ovum. Our studies are not as complete as they might have been, had we not been compelled to drop them at an early date in order to prepare for



examinations and graduating exercises. But we have both arrived at the same conclusion, viz. *that the ovum does have an epithelial origin*. In regard to the mode of origin, we have arrived at conclusions differing somewhat from any that have yet been published. As before stated, the germinal epithelium forms a complete investment of the ovary, in the form of flat polyhedral cells in a single layer. In surface views (fig. 17) these cells are seen to differ considerably in size in different areas, being generally smaller in those places where newly formed ova are found, or where such are in process of formation. The difference in size, however, is not so great as surface views might lead one to think. In sections passing through such areas (figs. 13 and 14) the cells appear more crowded than elsewhere, and have greater depth. In the least crowded areas the nuclei have a flattened elliptical form, when seen in section, and are placed at considerable distances apart.

In the more crowded areas the nuclei are brought so close together that the cell protoplasm is not always to be recognised between them, and they are tilted up on one end, so to speak, and often overlap one another (fig. 14).

It is in these crowded areas that we meet with the germ-cells,<sup>1</sup> which lie at first *in* the germinal epithelium (fig. 15), and are plainly a part of it. Such a section as that given in fig. 15 would perhaps, at first sight, be supposed to favour the view that the germinal epithelium is composed of two distinct kinds of cells. There are some points in this section, however, that will be more easily explained after a description of surface views.

I have found very often during the winter the nuclei of the germinal epithelium in process of division, as seen in fig. 2. Other cells of the same layer are found with two distinct nuclei (figs. 11 and 12). The two nuclei may be alike in general appearance (fig. 11), or one may present an aspect quite unlike that of the original nucleus. The latter case has been reproduced in fig. 12, in which four epithelial cells are represented. In one cell there is seen, besides the proper nucleus, which is precisely like the nuclei of the neighbouring cells, a second somewhat larger body, which is quite distinctly outlined, and which has a nucleus-like body within. That this body lies wholly within the epithelial cell there can be no doubt. This body represents a germ-cell which has formed within the parent epithelial cell.

The question at once arises, How does this endogenously

<sup>1</sup> As I am uncertain whether these cells give rise to both the primordial ova and granulosa, I call them germ-cells.



formed cell escape from the parent cell? By comparing the surface views represented in figs. 3, 6, and 7, it will be seen that the nucleus destined either to be, or to give rise to, the nucleus of the ovum, increases in size and becomes coarsely granular. In fig. 3 it occupies about one half of the original cell, and that end of the cell in which it is lodged is bounded by a curved line. The second nucleus of this cell differs in no particular from those of surrounding cells, except that it has a concavo-convex form, which it has probably assumed in adjustment to the larger nucleus. In this case I found no well-defined body of protoplasm enveloping the larger nucleus, as in figs. 5, 6, 7, and 12. In fig. 7, which represents a single epithelial cell with a rounded outline due to the action of acetic acid, the larger nucleus is surrounded by a distinctly marked area of protoplasm, in close apposition with which lies the smaller nucleus, which has here nearly the same form as in figs. 3 and 6.

In fig. 6 the larger nucleus, together with its protoplasmic envelope, has taken the first step towards freeing itself from the mother-cell. It is still partly within the original cell, but dips at one end under two other epithelial cells. It is simply the expansion by growth of the young germ-cell, and not any active amœba-like movement, which causes it to get under the neighbouring epithelial cells and beyond the limits of the mother-cell. In some cases I find the boundary of the germ-cell passing directly into that of the original cell, always so when the dipping just begins, as seen in fig. 1.

The germ-cell at this stage has no membrane, although its protoplasm is clearly bounded. In the minds of those who hold a different opinion in regard to its origin, fig. 6 may be said to admit of another interpretation. It may be said that the germ-cell possibly lies wholly under the germinal epithelium, having no connection with it whatever. This mode of explanation was the first to occur to me, and I have endeavoured to test it, not only by the study of a very large number of surface preparations, but also by sections. I find it quite impossible to reconcile this view with my observations. Sections confirm in a very positive manner the explanation which I was led to adopt from the study of surface views. The youngest germ-cells are always found in the epithelial layer. Two of these are seen in fig. 15, one of which (on the left) corresponds exactly with what is seen in fig. 7. On the left of the germ-cell is a nucleus, elongated in a vertical direction,

lying in close contact with the germ-cell, and to all appearances answering to the concavo-convex nucleus of fig. 7.

In fig. 6 the germ-cell has already begun to get under the neighbouring epithelial cells. This section shows also that the epithelial layer thickens around the growing germ-cell, the epithelial nuclei being more crowded and sometimes lying beneath the surface. This crowding of the nuclei around the germ-cell is seen in all cases, and is well illustrated in figs. 4, 5, and 17. Fig. 6 shows what I have often met with, the nuclei of the surrounding epithelial cells lying each in that side of their respective cells which is in contact with the proliferating cell.

In regard to the origin of the follicular epithelium, I have not been able to come to any definite conclusion. I am quite certain that the germinal epithelial cells cluster about the germ-cell, and that they form a more or less perfect envelope around it, as is seen in the right germ-cell of fig. 16. In fig. 9, outlined with the camera from a preparation in Kleinenburg's fluid, are seen some small germ-cells, around which are clustered epithelial nuclei, and a young ovum invested with what I regard as the young follicular epithelium. The epithelial nuclei around the germ-cells differ in no respect from those around the ovum. In fig. 18 is seen a section of an ovum of about the same size, around which are seen five nuclei, belonging to as many cells, whose limits are not recognisable. On the inner side of this ovum (lower side in the figure) three distinct layers are recognisable, the lining epithelium of the ovary, the stroma, and the follicular epithelium; while on the outer side only two layers are to be seen, the germinal epithelium and the follicular epithelium. In an ovum having a diameter about two and a half times that of fig. 9 (fig. 19) we find the same three layers on the inside (left side of the figure) and one additional layer (stroma) on the outside. The stroma layer has now become very thin, and the nuclei of both the follicle and the lining epithelium are much smaller than in fig. 9.

It would be easy to interpret all this in favour of the opinion that the granulosa takes its origin from the germinal epithelium. But I am not prepared to deny Nussbaum's theory, according to which both the granulosa and the primordial ovum are derived from the germ-cell. I have found germ-cells with multiple nuclei, as seen in figs. 9 and 10; but I have not succeeded in ascertaining the fate of these nuclei. I have never found a "mulberry stage" of the nucleus in germ-cells as large as the ovum seen in fig. 9. I think

there can be little doubt that some of the nuclei seen on and around the ovum in fig. 17 correspond to the follicular nuclei seen in fig. 18.

The nuclei around the ovum (fig. 19) are precisely like those around the small germ-cells, and these nuclei are to be found around the germ-cells which have multiple nuclei, as well as around those with simple nuclei. While thus my observations incline me to the theory of the epithelial origin of the granulosa, I cannot regard them as conclusive.

The origin of the ovum, according to the above observations, may be briefly stated in the following words :

A nucleus of a germinal epithelial cell divides into two ; one remains as the nucleus of the proliferating cell, the other enlarges and becomes the centre of a well-defined portion of the protoplasm belonging to the parent-cell. The germ-cell thus formed lies at first wholly within the parent-cell, but as it increases in size it expands beyond the limits of the latter, and sinks beneath the surface, becoming at the same time free from the mother-cell.

Epithelial cells crowd around the germ-cell, and some, sinking beneath the surface with it, form a follicular (?) wall around it. The germ-cell represents a primordial ovum, or a cell from which the primordial ovum plus the follicular epithelium arise.

#### *Formation of Yolk-spherules.*

In the young germ-cells the protoplasm is clear and transparent, like that of the epithelial cells. The first plain indication of the presence of yolk-spherules appears about the time the egg has attained a diameter of .25 mm. At this time the ovum appears as represented in fig. 20.

The protoplasm has become somewhat clouded with very minute granules ; and scattered patches, consisting of larger or smaller aggregations of small yolk-spherules, are seen on one side of the ovum lying near the periphery. These aggregations are quite opaque, although the single spherules are not so. Viewed singly, after being expelled by pressure of a cover from a ruptured ovum, they appear to be minute shining spherules, measuring about .001 mm. They have a refrangibility much like that of particles of fat, and show the usual Brownian movement. Along with aggregations numbering from 20—25 spherules are found others consisting of only two or three. The spherules of the patches do not vary much in size ; but outside these patches are found single spherules, varying from the size of those in the



patches to that of the most minute granule that we are able to recognise.

Seen by reflected light these masses of yolk-spherules appear whitish. These patches are found at a little later date scattered throughout the peripheral portion of the ovum, but a little larger and more closely arranged on that side of the ovum where they presumably first appeared than on the opposite side, as seen in fig. 22.

In this section of an ovum, measuring .57 mm. in diameter, and hardened in picro-sulphuric acid, the yolk patches present an appearance quite different from that which they have when examined in a fresh condition. The fine granular protoplasm here appears darker than the patches themselves—just the reverse of what is seen in the living egg. The size of the spherules is now .002 to .003 mm. In fig. 21, which represents an optical section of a portion of an ovum measuring a little less than 1 mm., examined in a fresh condition, some of the aggregations appear to be more or less elongated in a radial direction. The further history of these deutoplasmic masses can be learned only by the study of sections of hardened ova; the protoplasm stains deeply red in picro-carmin, and the same is true of the narrow boundary lines between the radially elongated masses of yolk-spherules. The deutoplasmic elements and the protoplasmic matrix are thus well defined by the action of the staining fluid. The persistence of the bounding lines even after the greater portion of the ovum has become thickly crowded with yolk-spherules is quite remarkable, and I am not aware that such a feature has been described before. That it is not an artificial product is perfectly certain, for I constantly meet with it in eggs varying in size between .5 mm. and 1 mm., and meet with it in all phases intermediate between figs. 20 and 23, and between fig. 23 and later stages of complete obliteration.

The disappearance of these radial bounding lines occurs soon after the egg has attained a diameter of 1 mm., some time *before* the yolk masses have reached, in their inward growth, that stratum of protoplasm which lies next to the germinal vesicle. The latter event is usually accomplished by the time the ovum has attained a diameter of 1.5 mm.

That these young yolk-spherules are not cells is of course quite certain.

That they originate *within* the protoplasm is proved by the fact that there is always a cortical zone entirely free from them, and that no such corpuscles are to be found at any time outside the protoplasm. That they arise in the



manner described by Gegenbaur (13) seems most probable.<sup>1</sup>

The persistence of bounding lines, by means of which the individuality of the yolk masses is maintained for a remarkably long time, makes it clear that there is a centripetal growth of these masses. It is probable—we may say certain—that this growth is due, not only to the expansion of the individual spherules, but also to the formation of new spherules, which are added to the mass on the central side.<sup>2</sup> Just before the complete obliteration of the boundary lines of the yolk aggregations, the entire body of deutoplasmic spherules may be said to be made up of a considerable number of pyramidal masses, the bases of which lie in the subcortical zone, while the apices lie in the perinuclear zone. The basal portions of these pyramids are formed first, and the apical portions, which are farthest removed from the nutritive supply, are the latest to develop. It should be added, however, that the boundary lines never extend to the innermost, last formed, zone of spherules; hence the outlines of the yolk pyramids are never complete.

The distribution of the earlier formed yolk-spherules in small masses or aggregations in the subcortical zone has been observed by Götte in *Bombinator igneus* (7, p. 17); by Balfour (14, p. 410) in *Scyllium canicula*; and by His (15, p. 31, figs. 32 and 40, pl. iv) in Salmon.

The form of the yolk-spherules is from the outset ellipsoidal. In the mature egg the larger spherules measure  $\cdot 011$  mm. by  $\cdot 017$  mm.; they are clear and homogeneous, showing nothing which could give any support to the idea that they are of a cellular nature. The cortical layer of protoplasm, which has a thickness of  $\cdot 007$  mm. in fig. 23, becomes very thin in the mature egg, but does not wholly disappear. In fig. 26, which represents a ripe follicular egg, the yolk-spherules have encroached much upon the cortical layer, and here, as in earlier stages (figs. 24 and 25), the size of the spherules diminishes in the peripheral portion.

#### *Vitelline Membrane.*

As before remarked, the primordial egg-cell is membraneless. Nothing in the form of a membrane appears for some time after the ovum becomes enclosed in a proper follicular

<sup>1</sup> Kolessnikow (12, p. 400) makes a slip in saying that Gegenbaur refers the origin of the vitelline spheres to the follicular epithelium.

<sup>2</sup> I have seen nothing to favour the idea suggested by Götte (7, p. 18), that the first formed spherules move on toward the centre of the ovum, and that their original places are occupied by newly formed spherules.

epithelium. In all ova under 1 mm. in diameter, the cortical layer of protoplasm follows closely the boundary of the cells of the granulosa, as seen in fig. 21.

In ova measuring about 1 mm. may be seen a thin superficial portion of the cortical layer, quite transparent and free from granules (fig. 24). The outer contour of this thin layer is sharply defined, but there is as yet no line of division between it and the cortical zone. This surface stratum of the cortical layer, which is about .001 mm. thick, is the vitelline membrane in process of formation. In an ovum of 1.5 mm. (fig. 25, *v. m.*) it has taken the form of a double-contoured membrane, which appears to be perfectly homogeneous. In this section, which was treated with chromic acid ( $\frac{1}{3}$  per cent.) and Beal's carmine, the membrane was stained more deeply red than the underlying protoplasm. There is no doubt in my mind that the membrane seen in fig. 25 is identical with the clear surface stratum in fig. 24; and as in the latter figure it is continuous with the protoplasm, I regard it as a modified part of the protoplasm, and hold that the vitelline membrane is produced by the egg itself, and not by the granulosa.

### *The Germinal Vesicle.*

My observations on the germinal vesicle, especially on its earlier stages of growth, are quite incomplete. In ova measuring about .26 mm., examined in a fresh condition, the germinal vesicle appears perfectly clear, is spherical, and has a somewhat excentric position (fig. 20).

Numerous germinal dots (.003 mm.) lie at the very surface of the nucleoplasm (Van Beneden), apparently in contact with the inner surface of the very thin delicate membrane of the vesicle. In ova hardened in picro-sulphuric acid and stained with hæmatoxylin the nucleoplasm has a reticulate appearance (figs. 19 and 27). Cases like that seen in fig. 19 occur not infrequently, in which the nucleoplasm has contracted more than the membrane, in which case the germinal dots remain adhering to the membrane. In other cases the membrane follows the contracting nucleoplasm, as seen in fig. 27. In fig. 22 is seen a germinal vesicle, of about the same size as that seen in fig. 27. The ovum from which fig. 27 was taken corresponded also very nearly in size with the one given in fig. 22. In fig. 22 the germinal vesicle appears to lie in a large cavity, or rather to be suspended from one side of the cavity. This cavity, if it existed in the living egg, was undoubtedly filled with a watery fluid, upon which the staining fluid had no visible effect. In fig. 27 the

vitellus lies in close contact with the germinal vesicle, except at one side, where a small space, probably caused by contraction, is seen.

That the germinal vesicle in fig. 22 filled the whole cavity in the living ovum is very improbable, since it is not at all abnormally small—in fact is about equal to the germinal vesicle in fig. 27.

In fig. 22 the nucleoplasm is a little more coarsely granular than the egg-protoplasm, except the peripheral portion, which contains the nucleoli, and which is less coarsely granular than the central portion.

The germinal vesicle has everywhere except on the side of contact a sharp outline, but there does not appear to be a double-contoured membrane.

On the side of contact is seen an elongated area of perfectly homogeneous, non-granular, deeply-stained substance, which extends a little beyond the limits of the germinal vesicle in both directions. In this substance are seen two or three of the germinal dots, and the substance is continuous with the nucleoplasm, from which it differs only by having no granules. On the side of contact there is certainly nothing that could be called a membrane. It seems probable that this non-granular substance is a part of the germinal vesicle, and that the germinal vesicle itself is upon the eve of certain internal changes, some of which are seen in fig. 28. This germinal vesicle (fig. 28), belonging to an ovum measuring .82 mm. in diameter, is still more flattened than in fig. 22, and the outline is less regular, and shows no sign of a membranous envelope. The germinal dots have taken on peculiar forms, and appear to be moving towards the centre of the vesicle. Several elongated forms are seen, some of which are constricted about the middle, evidently in process of division. In some cases the division is just on the point of completion, the two halves still being joined by only a slender thread.

Another peculiarity, not observed in earlier stages, is the vacuole-like space seen in most of the germinal spots. In the elongated forms generally two such vacuoles, located at the two poles, are seen, and sometimes the same number is seen in the non-elongated massive forms. Hertwig (16) has described similar appearances in the egg of *Hæmopsis* and in that of the Frog, and v. la Valette St. George (17, p. 58) has observed the same in the egg of one of the *Libellula*.

Together with this breaking up and centripetal movement of the dots there is also a concentration of the coarser



nucleoplasmic elements, leaving a wider peripheral portion than is seen in fig. 22, containing the less granular part of the vesicle.

In fig. 29 these changes are carried still further. The germinal dots have been reduced by division to coarse granules of various sizes, and have collected in a central mass, leaving a broad peripheral portion almost as homogeneous as the small elongated mass at one side of the vesicle in fig. 22.

Scattered dots are seen in this peripheral portion. The germinal dots appear to have undergone some change in composition, judging from the effect of the staining fluid.

In fig. 28 the nuclear substance is stained, but much less strongly than the dots. In fig. 29 the nuclear substance is coloured as before, but the fragments of the dots have a dull yellowish-brown colour instead of the deep red of fig. 28.

In fig. 29 the vitellus is in immediate contact with the germinal vesicle, the clear space seen in fig. 22, which was also present in fig. 28, having entirely disappeared.

Owing to the limited amount of time which I have had to devote to this part of my study, I have not been able to learn the further course of events in the germinal vesicle.

### *The "Yolk-nucleus."*

In small ova, measuring from .15 mm. to .16 mm., I have several times met with an oval body at one side of the germinal vesicle (fig. 8). I do not find this body in all eggs of this size; indeed, it may be said to be a rather rare occurrence. This is undoubtedly the *Dotterkern* (not to be confounded with the "Dotterkern" of Goette) of German authors.

Hertwig (16, p. 37) has proposed to designate this body as "*Dotterconcrement*," in order to avoid the comparison implied in the term "yolk-nucleus." I can say nothing in regard to the origin or fate of this body, but as it is not constant, no special importance can be attributed to it. It has a granular composition, but this fact does not warrant the opinion that it is a mere aggregation of yolk-granules.

Van Bambeke refers the origin of this body to the follicular epithelium, one of the cells of which gets loosened and embedded in the ovum.

Waldeyer (4, p. 75) remarks that this body is found only in young ova; later it appears to disappear completely.

Leuckart (18) and others have made similar observations.



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1. *Salamandra suberistata*. Temminck et Schlegel, 'Fauna Japonica,' Reptilia.
  2. *Bambecke*, "Nouvelles Recherches sur l'Embryologie des Batraciens," 'Archives de Biology,' t. i, fas. ii, 1880.
  3. *S. F. Clarke*, 'On the Development of *Amblystoma punctatum*,' part i, External, 1879.
  4. *Waldeyer*, 'Eierstock und Ei,' Leipzig, 1870, p. 74.
  5. *Van Beneden*, 'Recherches sur la Composition et la Signification de l'Œuf,' 1868.
  6. *Ludwig*, 'Ueber die Eibildung im Thierreich,' 1874.
  7. *Götte*, 'Die Entwicklungsgeschichte der Unke,' 1875.
  8. *Nussbaum*, "Zur Differenzirung des Geschlechts im Thierreich," 'Arch. f. Mikr. Anat.,' xviii, p. 1, 1880.
  9. *Falaoritis*, "Ueber die Genesis beim Land-Salamander (*S. maculata*)," 'Zool. Anzeiger,' 1879, No. 42, p. 597.
  10. *Spengel*, "Das Urogenitalsystem der Amphibien," 'Semper's Arbeiten,' iii, p. 63.
  11. *Brandt*, "Fragmentarische Bemerkungen ueber das Ovarium des Frosches," 'Zeitschr. f. Wiss. Zool.,' xxviii, p. 584.
  12. *Kolessnikov*, "Ueber die Entw. bei Batrachiern und Knochenfischen," 'Arch. f. Mikr. Anat.,' xv, pp. 397, 403, and 410.
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  14. *Balfour*, "On the Structure and Development of the Vertebrate Ovary," 'Quart. Journ. of Micr. Sci.,' vol. xviii, 1878.
  15. *His*, 'Untersuchungen ueber das Ei und die Eientwicklung bei Knochenfischen,' Leipzig, 1873.
  16. *O. Hertwig*, "Beiträge z. Kenntniss d. Bildung, Befruchtung u. Theilung d. Thier-Eies," 'Morphol. Jahrbuch.,' vol. iii, 1877.
  17. *V. la Valette St. George*, "Ueber d. Keimfleck u. d. Deutung d. Eitheile," 'Arch. f. Mik. Anat.,' ii, 1866.
  18. *Leuckart*, 'Handwoerterbuch der Physiologie,' vol. viii, article, "Zeugung."
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*The GERMINATION and EMBRYOGENY of Gnetum Gnemon.*

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*Historical.*

THOUGH the structure of the ripe seed in the genus *Gnetum* is well known, and has often been described,<sup>1</sup> our knowledge of the development of the embryo-sac, with its contents, and of the embryo, is still very incomplete. Strasburger ('Ang. und Gymnosperm.,' p. 116) has succeeded in tracing that of the ovule with its several embryo-sac mother-cells, and the first stages of development of the embryo-sac in *Gnetum Gnemon*. But here our information stops short, the further growth of the embryo-sac and of its contents not having been followed with accuracy. In the mature seed the embryo-sac is found, as in other Gymnosperms, filled with endosperm; towards the apex of this is usually formed a cavity, which in some species is filled with a coiled mass of tubular structures; this mass was described by Blume (l. c.) and by Griffith (l. c.) as a funiculus (suspensor in present terminology). In some species (*G. scandens*, by Griffith, and *G. latifolium*, by Blume) an embryo has already been observed borne on the end of the coiled suspensors; it has two small cotyledons, and the figures given by these authors correspond generally with certain stages of development to be described below (cf. fig. 10). Further, in the description of the characters of the genus *Gnetum*, it is stated by Parlatore ('Prodromus de Candolle,' xvi, pt. ii, p. 349), and by Tulasne (Martius' 'Flora Bras.:' vol. iv, pt. ii, p. 399), that the embryo is embedded in endosperm,

<sup>1</sup> Blume, 'Ann. Sci. Nat.,' 1834; and 'Rumphia,' Bd. iv, taf. 176, &c.; Roxburgh, 'Flora Indica,' vol. iii, p. 519; Griffith, 'Trans. Linn. Soc.,' vol. xxii, p. 299; Hooker, 'Trans. Linn. Soc.,' vol. xxiv, p. 39; Strasburger, 'Coniferen and Gnetaceen,' pp. 101, 236; Beccari, 'Nuovo Giornale Botanico Italiano,' vol. ix, p. 91; Strasburger, 'Angiospermen und Gymnospermen,' p. 100.

and has two small cotyledons, &c. The latter author, however, gives drawings of seeds with a cavity in the endosperm but no embryo. Finally, in the published account of the botanical results of the voyage of the Coquille (1822—1825), seeds of *Gnetum Gnemon* are figured with an embryo.

Following a reference in Griffith's paper, above cited, I find that the embryo of *Gnetum scandens* had already been drawn (but not published) by Roxburgh, and on examining the original drawing in the Kew Herbarium, I find the embryo represented as having two unequal cotyledons of large size, a short radicle, and a plumule as long as the cotyledons, bearing at its apex two very small leaves; the suspensor is omitted. It will be subsequently seen that this does not tally with my observations on *Gnetum Gnemon*.

In other species (*G. Gnemon*) there is usually found a cavity near the apex of the endosperm of the mature seed, but no suspensors are apparent till sections are examined under the microscope, when it is found that tubular cells permeate the apical part of the endosperm. This fact has already been recorded by Sir J. Hooker (l. c.), who also noted that these tubes occasionally branch (cf. *infra*). In specimens of *Gnetum Gnemon* from Java, which apparently included all stages of development of the flowers, Strasburger ('Angiosp. und Gymnosp.,' p. 101) found no such bodies.

### *Ripe Seed.*

Early in 1881 I received from Java, through the kindness of Dr. Treub, two parcels of ripe seeds of *Gnetum Gnemon*. On examining longitudinal sections of the endosperm of these seeds, it is seen that there is no embryo already developed; the main body of the section consists of ordinary cells of the endosperm, loosely aggregated, with intercellular spaces between them; near its apex there is usually a cavity (but in specimens of *G. Gnemon* from the Kew Museum this is not always the case). The cavity is evidently due to rupture of the tissue, and its occurrence may depend upon the manner of ripening of the seed. Among the cells of the endosperm may also be found numerous long tubular cells, with walls which stain blue with solution of I in KI; they have rather transparent protoplasm, and a nucleus (I have never observed more than one nucleus); transverse septa occur in rare cases, but there does not appear to be any definite terminal cell cut off before germination. The course of the tubes through the endosperm is sinuous, and for the most part longitudinal; where they

traverse the cavity of the endosperm their course is more direct, and here cases of branching may occasionally be observed (this is described also by Sir J. Hooker, l. c.). The tubes may be traced backwards towards the apex of the endosperm and up to certain shrivelled bodies, which correspond in position and form (as far as could be judged in their disorganised condition) to the corpuscula of *Ephedra*. Whether this be their real nature remains to be decided by comparison with younger stages of development. We may for the present assume that they are the corpuscula, and apply that term to them, while we call the tubular cells suspensors.

Seeds of an unnamed species of *Gnetum* from Chittagong, preserved in the museum at Kew, were also examined; here each one of the suspensors does not, as in *G. Gnemon*, pursue a separate course, but they together form a coiled bundle which lies in the cavity of the endosperm, and can easily be removed with a needle (fig. 1). If the suspensors be teased apart, it is seen that each consists of a long tubular cell having the characters above described; the protoplasm increases in density towards the end of the tube, at some distance from which is embedded a single nucleus (fig. 2).

This species would doubtless be better fitted for the study of the development of the embryos than *G. Gnemon*, as they could thus be easily removed from the endosperm, whereas in *G. Gnemon* the only method possible for obtaining preparations of the youngest embryos is to cut sections of the endosperm, and search for the embryos, which are often found to be fixed in positions unsuitable for observation. Hence the study of the early stages of the embryo of *G. Gnemon* is by no means easy, and this will explain the incompleteness of my series of figures.

#### *Germination.*

The two parcels of seeds sent from Java were sown at Kew respectively in March and June, 1881. The first seedling of the first parcel appeared above ground early in November, eight months after sowing. The period of germination is, however, variable in different individuals. Owing partly to this fact, and partly to the difficulty in obtaining suitable preparations, I have not succeeded in observing the first changes at the apex of the suspensor. The young embryos are usually found near the axis of the endosperm, but at a very variable distance from its apex. Though the arrangement of cells in the youngest embryos which I have observed points to an origin from a single cell cut off from



the apex of the suspensor, I have not seen any specimen of this, excepting one doubtful case. This cell divides by anticlinal<sup>1</sup> walls, of which none appear to be exactly median (figs. 3—7).<sup>2</sup> The peripheral cells of the group thus formed grow laterally along the surface of the suspensor, and, dividing further by anticlinal walls, form short embryonic tubes, comparable to those of *Welwitschia*, though much less developed than these (cf. Strasburger, 'Angiosp. und Gymnosp.,' p. 155). The whole embryo now presents the appearance of a single layer of cells covering the apex of the suspensor like a hood (fig. 5). A cell is next cut off from the suspensor by a transverse wall (fig. 8). Then follows longitudinal division of the cell thus formed, while in the lateral parts of the peripheral layer cells now begin to divide by periclinal as well as by anticlinal walls; one cell at the apex, however, divides by walls perpendicular to the outer surface of the embryo, and inclined to one another (figs. 6—9). This cell appears to be wedge shaped; in fact, we have to deal with a growth with an apical cell such as has been observed by Strasburger in the case of many embryos among the Coniferae ('Conif. and Gnet.,' p. 302, &c.), and in a rudimentary way occasionally in *Ephedra* ('Ang. and Gymn.,' p. 154). By this mode of increase of the peripheral tissue, and by divisions both longitudinal and transverse of the central group of cells, a large embryonic body is formed, in the apical part of which the tissues differentiate in a manner similar to that well known in other members of the group. Internally a root-apex is formed, while externally there appears at the apex of the embryonic body two cotyledons and a central apical cone (fig. 10). A definite epidermis covers the cotyledons and the hypocotyledonary stem, but, as is the rule in the Gymnosperms, the root has no such covering; the same is the case with the conical apex of the stem; here the peripheral cells frequently divide by periclinal walls, while the extreme apex is not uncommonly occupied by a single large cell (fig. 13). For the sake of comparison, longitudinal sections were also cut through the apical cone of branches of an old plant growing in the Royal Gardens at Kew; though many preparations were made, including also the axillary buds, no undoubted case of division of a peripheral cell by a wall parallel to the surface

<sup>1</sup> I use this term in the same sense as Sachs, 'Ueber die Anordnung, &c.,' Würzburg, 1877.

<sup>2</sup> Compare the embryo of *Welwitschia*, as described by Strasburger ('Conif. und Gnet.,' p. 314). There the first embryonic cell is divided crosswise, and no apical cell is formed.

was observed (fig. 14). It must be noted, however, that these observations were made on buds dormant in winter. It is possible that at periods of great activity such divisions may occur, and this has been observed by Strasburger during the development of the ovule of *G. Gnemon* ('Ang. and Gymn.,' Pl. XI—XIV).

The form of the embryo and the mode of arrangement of the procambium bundles will be seen in fig. 10. The growth from this point onwards is very rapid. Shortly after the embryo has reached the stage represented in fig. 10 a lateral protuberance appears on the hypocotyledonary stem (fig. 11), the position of which is constant as regards the direction of gravity, *i. e.* it always appears on the under side of the embryo, as the seed lies during germination; its relation to the position of the cotyledons is, however, inconstant, the planes in which they are expanded having no direct relation to the direction of gravity.

Externally this outgrowth has a rounded apex, and as its development proceeds it assumes an approximately cylindrical form. It arises through a lateral extension of the tissues of the hypocotyledonary stem, the epidermis, cortex, vascular bundles, and pith all taking part in its formation. It is the morphological equivalent of the structure already described under the name of "Feeder," as appearing laterally at the base of the hypocotyledonary stem of *Welwitschia* ('Quart. Journ. Micr. Sci.,' vol. xxi, new ser.); there is, however, this difference between them: that in *Gnetum* the cortex and epidermis do not form the main bulk of the organ, as is the case in *Welwitschia*, but rather the pith and vascular bundles. The latter may be seen at an early stage curving outwards from the hypocotyledonary stem into the organ (figs. 11, 12), while in the fully developed feeder they extend almost to its extreme apex.

As in *Welwitschia*, the growth of the organ is rapid; it soon overtops the hypocotyledonary stem, and finally attains a length of about half an inch (fig. 15). As its length increases, its apex being fixed in the body of the endosperm, the weaker point at the micropylar end of the seed gives way; first the radicle and then the body of the embryo are gradually pushed out through the ruptured testa. Direct evidence of the pressure exerted on the apex of the feeder is afforded by cases such as that in fig. 16, where the end of the feeder is folded upon itself. Immediately on protruding from the seed the radicle curves downwards, while the suspensors and the apex of the nucellus remain attached laterally to it at a point near the level of the seed. Shortly

after the protrusion of the radicle the hypocotyledonary stem increases rapidly in length, and, curving upwards, gradually draws itself out from the cavity of the endosperm, leaving the seed below ground. The organ swells as soon as the hypocotyledonary stem is withdrawn from the endosperm; the space vacated by the stem is thus filled up, and the feeder comes into immediate contact over its whole surface with the endosperm.

The hypocotyledonary stem, which is pink, gradually becomes erect, and, elongating rapidly, attains a length of about six inches. The cotyledons, which have hitherto remained small (cf. fig. 17), now begin to expand and turn green; finally they assume the appearance of the ordinary foliage leaves of *Gnetum*, being petiolate with a thick midrib and reticulate venation. They sometimes exceed three inches in length. The stout primary root grows to a considerable length, and forms lateral roots at an early stage (fig. 18).

The plumule remains a simple papilla of tissue till the cotyledons begin to expand; it then forms two opposite plumular leaves decussating with the cotyledons, and these are followed by other pairs of leaves having a similar mutual arrangement. Soon after the plumular leaves appear, an axillary bud begins to be formed in the axil of each of the cotyledons, and these form opposite pairs of leaves in planes parallel to those of the plumule. Further, secondary axillary buds appear between the primary buds and the cotyledons. A similar arrangement occurs in certain species of *Ephedra*.

The same arrangement of parts is to be found in the ordinary buds of *G. Gnemon*, and in the old plant of this species growing at Kew axillary buds have developed into foliage shoots in a single instance on the strong main axis. Though this is of frequent occurrence in some species of *Ephedra* it seems to be the exception in *G. Gnemon*.

While these structures are young they are enclosed and covered in by swellings at the base of the petiole (fig. 19). Immediately above the young parts thus enclosed the peripheral tissues of these swellings are subject to a gummy degeneration of their walls, and the epidermis and part of the subadjacent parenchyma become disorganised; the place of the cells thus broken down is taken by fresh tissue derived from an actively dividing layer, which defines the glandular structure from the tissues beneath it. A mass of gummy substance is thus formed, which covers and protects the structures of the bud while growing. The first tangential



divisions in the parenchyma of the petiole preparatory to the formation of one of these glands may be seen in fig. 14. These glandular structures are found at the base of the petiole of the cotyledons, as well as of all foliage leaves.

### *Polyembryony.*

From what has been stated above it will be seen that polyembryony is the rule in *Gnetum*, in the same way as in other members of the group, viz. a number of suspensors are found in each seed (and probably several spring from each corpusculum); on the apex of each of these an embryo may be formed.

But with *Gnetum Gnemon* the matter does not end here. It has been above observed that branched suspensors occur occasionally in the ripe seed; and we have no reason to suppose that embryos may not be formed on the apex of each of these branches. I have, moreover, been able to observe directly that one suspensor can produce more than one embryo. In the case represented in fig. 20 a number of bodies, which resemble normal embryos in their structure, are borne on a single branched suspensor. Further, I can suggest no other explanation for irregular bodies, such as those represented in figs. 21, 22, which are not of unfrequent occurrence, than that they are instances of a like branching of suspensors (or perhaps proliferation of embryos, as it might be more correctly termed, in the case of fig. 22) seen at an earlier stage.

Besides such cases as these it very frequently happens that embryos are united by their posterior part, without its being possible to trace them back to the single suspensor (or, possibly, the suspensors) from which they originated; and in considering these cases it must be remembered that in *G. Gnemon* each suspensor, as a rule, pursues an individual course, otherwise this observation would have no bearing upon the point under consideration. It must remain an open question whether these embryos are simply in juxtaposition or are genetically connected. However this may be, the fact is demonstrated by instances, such as those in figs. 20 and 22, that there is to be found in some cases in *G. Gnemon* a higher order of polyembryony than that described for others of the Gymnosperms.

In each seed only one embryo becomes finally developed. The others, though rich in protoplasm in their early stages, lose their activity after a time; they become transparent, and are often squeezed out of shape; in fact they are aborted.



Such embryos were figured by Griffith ('Linn. Trans.,' vol. xxii).

*The Vascular System of the Seedling.*

From each of the cotyledons five vascular bundles enter the stem<sup>1</sup> (fig. 23); these curve downwards, and for a short distance pursue an individual course. Soon the marginal bundles of the two leaf systems fuse (fig. 23 b, bundles marked x), the whole number of bundles being thus reduced to eight. Further down the hypocotyledonary stem small branch bundles are formed, which run parallel to the larger bundles, while the latter occasionally fuse laterally. Thus the number of bundles found in transverse sections of the lower part of the hypocotyledonary stem is not constant. The arrangement of the bundles in a ring is maintained down the hypocotyledonary stem till the level of the feeder is reached. Here those bundles which are on that side of the stem on which the organ is curve outwards from the ring and pass into the feeder, leaving some three or more bundles behind in the stem; these pursue a direct course downwards into the root (figs. 24, 25). The bundles which enter the feeder branch repeatedly, but run with an almost direct course along the upper side of the organ; close to its apex they curve sharply, and returning along its under side re-enter the axis of the seedling. A transverse section of the feeder shows a ring of bundles (fig. 26), in which it is often difficult to distinguish the outgoing from the returning system. This is due partly to the branching of the bundles, partly to the formation of lignified elements between them, through the activity of a feeble interfascicular cambium, which appears at a comparatively late stage, and completes the vascular ring.

It was above stated that three or more bundles pass directly from the hypocotyledonary stem to the root. Some three more return from the feeder and rejoin these; the six or more bundles then pass downwards together into the root. Of these bundles, two appear stronger than the rest (fig. 24), and the xylem of these may be traced downwards, and, after rotation, so that the protoxylem takes a peripheral position, they are seen to be continuous with the two primary xylem masses of the root. The phloem of each of these bundles divides into two parts, which unite to

<sup>1</sup> Strasburger ('Conif. und Gnet.,' p. 115) has observed a similar number as supplying the foliage leaves of an unnamed species of *Gnetum*; the number varies, however, in different species. I have also observed seven bundles passing from each of the older leaves of *G. gnemon* into the stem.

form the two primary phloem masses of the root. The other bundles pass downwards in their original position, their phloem merging with the phloem of the root, while their xylem ends blind in the cambium of the root (figs. 24, 25).

The position of the feeder relatively to the plane, in which the two larger bundles and the primary xylem groups of the root run, is not constant. In 24,  $a-c$ ,  $d-f$ , are represented two extreme cases; in the one case ( $d-f$ ) the plane in which these bundles run coincides with the median plane of the feeder; in the other ( $a-c$ ) it is at right angles with the median plane of the feeder; in other cases the planes were found to cut one another obliquely. Whether the relative position of the cotyledons and of the primary tissues of the root is constant I have not been able to determine, but have no evidence to the contrary, and it must be remembered that in allied plants (*Welwitschia* and *Ephedra*) it is constant.

The inconstancy of the position of the feeder relatively, on the one hand to that of the cotyledons, and on the other to that of the tissues of the root, coupled with the constancy of its position on the under side of the embryo in the germinating seed, shows us (1) that the direction of gravity has a direct influence upon the point at which it appears, while the relative position of the parts of the embryo has not; and (2) that the direction of gravity has in this case no determining influence upon the position of the parts of the embryo.

The bundle sheath appears first about the same level as the lower side of the feeder, and on the opposite side of the axis to that structure (fig. 24, *b*) it encloses a pericambium several layers of cells thick. The primary structure of the root resembles that of *Welwitschia*, &c., and need not be described in detail. The primary xylem masses fuse centrally at some distance below the feeder, but parenchyma is interspersed between the elements.

As in *Welwitschia*, the cuticularisation of the bundle sheath begins on the radial walls, and spreads later to the tangential walls. At points opposite the primary xylem masses, however, the change in the tangential walls is deferred, and these cells of the bundle sheath, together with some cells of the cortical tissue, take part with the pericambium in the formation of the lateral roots<sup>1</sup> (cf. 'Conif. und Gnet.,' p. 348).

<sup>1</sup> This observation led me to study afresh the development of the lateral roots of *Welwitschia*. I find that my former opinion ('Quart. Journ.

Hitherto we have considered the structure of the seedling before the development of the plumule. In plants in which the first plumular leaves are already formed a system of vascular bundles similar to that of the hypocotyledonary stem is found in the first internode of the plumule; as they pass downwards the bundles branch, and coalesce laterally at the point of insertion of the cotyledons. Of the resulting bundles, which are reduced by successive lateral fusions to a small number, some insert themselves upon the bundles of the cotyledonary system, others (usually four) continue, for some time at least, a separate course between the cotyledonary bundles.

Meanwhile an interfascicular cambium appears in the hypocotyledonary stem, while the original bundles undergo secondary thickening; the vascular ring is thus completed. It is worthy of observation that there is an irregularity in the development of this ring immediately above the feeder; here, on the side of the stem opposite that organ, *i. e.* where there is a direct vascular communication with the root, the xylem is more strongly developed than on the side next the feeder; the reverse is the case, though in a less marked degree, with the phloem. These observations are of importance when connected with the fact that the seedling is dependent upon the root for its supply of water, and upon the endosperm for its supply of nutritive materials.

The secondary thickening of the vascular system of the root is derived from two cambium zones, similar in position to those of *Welwitschia*; in the oldest roots as yet investigated the activity of this cambium remains, while there is no formation of peripheral bundles as in *Welwitschia*. The same is the case with the stem.

### *Sclerenchyma.*

In the pith and cortex of the stem are found sclerenchymatous structures of various sorts: (1) such as are obviously derived from cells of the parenchyma, the form of which they retain; their walls are pitted and lignified; (2) cells with the same characters as the above, but with stellate form, each cell having formed one or more conical outgrowths, which follow the intercellular spaces; (3) towards the periphery of the stem (and root) are found long unbranched sclerenchyma fibres with smooth walls; when

*Micr. Sci.*, Oct., 1881, p. 577) is incorrect. In this plant also both bundle sheath and some cells of the cortex take part in the formation of the lateral roots.



mature these overlap one another at their ends, so as to form a continuous system. If such fibres in early stages of their development be examined, they are found to have two or more nuclei; the larger the number of the nuclei the less definite is their outline (fig. 26). Similar fibres occur in the petiole and lamina; in the latter they branch, and form a dense mat, permeating the intercellular spaces of the leaf immediately beneath the epidermis.

*Laticiferous vessels.*

In the parenchyma of the stem and leaves of *G. Gnemon* (but not observed in the root) are to be found laticiferous vessels, which appear when mature as tubes of considerable calibre, with cellulose walls, and contents having the characters of latex. The walls are smooth and of medium thickness; where two members of the vessel are contiguous at their oblique ends (fig. 27) the wall is more or less completely absorbed, and the contents of the originally separate cells are connected with one another.

On the lateral walls are found not uncommonly points where the wall is thin, and has bulged outwards as sac-like protuberances, which encroach upon the cavity of neighbouring cells; these thinner points in the walls are pitted (fig. 28), but not perforated (de Bary, 'Vergl. Anat.,' p. 195).

The latex, when coagulated with alcohol, appears under the microscope as an amorphous, highly refractive, yellowish substance. It swells with potash, but resumes its original appearance on acidulating. It stains a deep yellow with iodine or Schultze's solution; it resists strong sulphuric acid, retaining its sharp outline, and turns a dusky black after treatment with osmic acid. Highly refractive globular bodies, which give similar reactions, occur in the cells of the surrounding parenchyma.

It appears, then, that we have to deal with a latex of similar character to that of the Angiosperms.

The laticiferous vessels appear at a very early stage in the young seedling, and may be recognised while the feeder is still very small. Their development is, however, more easily traced in longitudinal sections of the bud of the older plant, and the following results are in the main obtained from this source.

The laticiferous vessels are derived from cells which are arranged with considerable regularity in longitudinal rows; such cells are to be found in the young stem and leaves, but not in the apical cone above the youngest



leaves. They are distinguished from the surrounding tissue by ceasing to divide; they soon attain an oblong form (fig. 29); the terminal walls are at first transverse, but as growth goes on they usually become oblique; the ends of the component cells of the vessels thus assume a similar relation to one another to that of the ends of the sclerenchyma fibres. The protoplasm of the cells when young resembles that of the surrounding tissue; but soon after the cells assume the oblong form the formation of the latex begins. Irregular masses of the substance similar to that contained in the mature vessels appear in the protoplasm of the cells (fig. 39); these increase in size as the development proceeds.

In several cases more than one nucleus has been observed in these cells (fig. 29), but this cannot be stated as a constant character. Still it is a fact of interest when connected with the development of the sclerenchyma fibres above described. And it may be remarked that, were it not for the early appearance of the latex in the young laticiferous vessels, it would be almost impossible to distinguish them from the young sclerenchyma.<sup>1</sup> Even when mature there is a close similarity between the two tissues in the form of their component elements, as well as in the position of those elements relatively to one another.

No great importance is perhaps to be attached to the fact that more than a single nucleus is found in some cases in the cells which form the laticiferous vessels, but in this point *Gnetum Gnemon* seems to differ from other plants having articulated laticiferous tissue.

In young laticiferous vessels are frequently to be seen attached to the lateral walls small masses of a substance which resembles the coagulum of latex in its properties, but is distinguished from it by its position. It is possible that these bodies may have some connection with the formation of the sac-like outgrowths of the walls above described, but this point has not been established.

A similar substance is also found in comparatively large masses on the walls which are about to be absorbed; here, as before, the proof that any constant connection exists between the appearance of this substance and the breaking down of the septum has not been obtained (fig. 30).

<sup>1</sup> A case like this appears as a strong excuse for the confusion of sclerenchyma fibres with laticiferous tissue by Mirbel and others. Comp. de Bary, 'Vergl. Anat.', p. 208.

*Germination of Ephedra.*

Seeds of *Ephedra monostachya*, and *intermedia* were sown in order to compare their germination with that of *Welwitschia*, and *Gnetum*.

Experiments were made in sowing the seeds in different positions as shown in the following table :

|                        | No. of seeds sown, and position. | No. of seeds that came up. | Seed brought up on apex of cotyledons.                                | Seed left below. |
|------------------------|----------------------------------|----------------------------|-----------------------------------------------------------------------|------------------|
| <i>E. monostachya</i>  | Radicle downwards 6              | 2                          | 2                                                                     | ...              |
| "                      | Sown on side . 6                 | 1                          | ...                                                                   | 1                |
| <i>E. intermedia</i> . | Radicle downwards 6              | 5                          | 2                                                                     | 3                |
| "                      | Sown on side . 6                 | 5                          | 1 { Abnormal :<br>cots. having<br>perforated<br>the endo-<br>sperm. } | 4                |

These experiments were confirmed by observation of a large number of seeds less carefully sown. Though these results cannot be regarded as conclusive, still it will be seen that in the large majority of cases the position of the seed during germination has a determining influence upon the relation of the seed to the growing seedling ; moreover, the difficulty in placing the seed with the axis of the embryo exactly vertical may account for the want of strict uniformity of these results with the conclusions drawn by Strasburger from the germination of *E. campylopoda* (' Conif. und Gnet.,' p. 321).

In those cases where the seed is carried up on the apex of the cotyledons the nutritive materials of the endosperm are completely absorbed by them.

In cases, however, where the cotyledons were withdrawn at an early stage from the seed, it was found that this took place while considerable quantities of starch and protoplasm still remained in the endosperm. In seeds which were examined at a later period of germination these nutritive materials of the endosperm were found to have been entirely absorbed.

There must therefore have been a transfer of these substances from the endosperm to the seedling after the cotyledons had been withdrawn ; and since the suspensors and remains of the nucellus maintain a direct connection between them, while the suspensors at least retain an appearance of vitality, we must conclude that the transfer is con-

ducted through these tissues. There is in the species of *Ephedra* above named no lateral outgrowth of the hypocotyledonary stem comparable to the feeder of *Welwitschia* or *Gnetum*, but it will readily be seen that, were the store of nutritive substances in the endosperm larger, as is the case in the other two genera, the need of some special provision for the transfer would be more pressing. Moreover, in the seedling of *Ephedra* we have a parallel process to that which is the chief function of the feeder. In both cases nourishment is received by the seedling, and absorbed from without at the surface of the base of the hypocotyledonary stem.

### *Conclusion.*

Though the development of the corpuscula and the process of fertilisation have not yet been observed in *Gnetum Gnemon*, it seems probable that in these respects it resembles *Ephedra*. But in *G. Gnemon* it is found that in the ripe seed the suspensors bear no embryo, appearing merely as more or less branched tubes, which permeate the apical part of the endosperm. On the germination of the seed these suspensors are capable of further development and of producing embryos, the seed itself being meanwhile independent of the parent plant.

A similar process has been described by Strasburger ('Conif. und Gnet.,' p. 295) in *Ginkgo biloba*, and by Warming in *Ceratozamia*, and some other Cycads ('*Bidrag til Cycadeernes Naturhistorie*,' French summary, 1879). It would be a mistake to attach much importance to this matter as a character in common between the groups in question (compare Heer, 'Bot. Centralblatt,' 1882, p. 239). The inconstancy of the phenomenon in each of the three groups is sufficient argument against such a view.

These cases may be compared with those Angiosperms in which the embryo is still rudimentary in the ripe seed.

The mode of development of the embryo in *G. Gnemon* corresponds closely to the type for the Gymnosperms. At an early stage an apical cell makes its appearance, and it may be recognised in much older embryos than is possible in *Ephedra*. In its appearance and the length of period of activity of its apical cell the embryo of *G. Gnemon* compares more closely with that of *Juniperus* or *Thuja* than with that of *Ephedra*. In *Welwitschia* according to Strasburger ('Angiosp. and Gymnossp.,' pl. xxii) there is no apical cell.

In the differentiation of the embryonic body *G. Gnemon*

resembles other Gymnosperms; at the apex of the stem the dermatogen is not well defined in young embryos, but in the mature plant there is apparently a more independent layer of dermatogen at the apex of the stem than is seen in *Ephedra*.

Soon after the cotyledons and apex of the root appear, and after the procambium bundles are differentiated, the feeder is formed by lateral outgrowths of the tissues of the hypocotyledonary stem. Though it is no doubt the homologue of the similar structure in *Welwitschia*, the two differ from one another in certain details. The vascular bundles of the hypocotyledonary stem, which in *Welwitschia* only curve slightly outwards at the level of the feeder, describe in *Gnetum* a strong curve, passing outwards along the upper side of the organ nearly as far as its apex, then curving sharply, they return along its lower side. Thus the arrangements for transfer of materials from the endosperm are more elaborate in *Gnetum* than in *Welwitschia*. Further, in *Welwitschia*, the seeds of which are flat, and the planes of the cotyledons parallel to that of the seed, I have already shown ('Quart. Journ. Micr. Sci.,' Jan., 1881) that the feeder may be formed at either of two points, the choice being determined by the direction of gravity relatively to the seed during germination. In *Gnetum* the seed is polysymmetrical; the direction of the planes of the cotyledons, which are small, and not much flattened at an early stage, bears no definite relation to the direction of gravity during germination. The position of the feeder is, however, determined by the latter, and the organ always appears on the under side of the embryo as it lies during germination. Hence it follows that the position of the feeder relatively to the vascular system of the stem and to the position of the cotyledons is inconstant in *Gnetum*, while in *Welwitschia* it is constant.

Comparing the seeds of the three genera of the Gnetaceæ and their several modes of germination, it is seen that they form a series in which the special adaptation for the transfer of nutritive materials from the endosperm to the seedling varies directly with the size of the seed, *i. e.* with the amount of nutritive material to be transferred. In *Ephedra* the endosperm is small, but still, in certain cases, the cotyledons leave the endosperm before the store is used up, and the further transfer has to be performed by the very inefficient tissue of the suspensors.

In *Welwitschia* the seed is larger, and, as before, the cotyledons leave the endosperm at a comparatively early stage;



there is, however, in this case, a special adaptation for transfer of the store material, the feeder being formed by which the seedling is connected with the endosperm. In this case the organ is composed chiefly of parenchyma.

*Gnetum* has the largest seed of the group. As before, the cotyledons soon escape from the seed, and a feeder is formed; not only is this larger than that of *Welwitschia*, but there is also found in it a peculiar arrangement of vascular bundles, the function of which is obviously to facilitate the supply of the nutritive materials of the endosperm to the stem and root of the seedling.

The feeder of *Gnetum* is, like that of *Welwitschia*, formed by lateral extension of tissues of the hypocotyledonary stem; the morphological value of the organ in both cases is that of an emergence. The feeder of *Gnetum* may be compared structurally with the complicated emergences on the exterior of the fruit of *Datura* and *Æsculus*, since in both cases vascular bundles enter them.

It has already been shown ('Quart. Journ. Micr. Sci.,' Jan., 1881) that the outgrowth of the tissues of the hypocotyledonary axis (feeder) of *Welwitschia* is equivalent to the foot described by Pfeffer in *Selaginella*, and it was there pointed out that those organs in these two genera are alike in their physiological functions, as well as in their structure and position; they differ slightly, however, in their time of appearance. A similar comparison may be drawn between the feeder of *Welwitschia* and the foot of *Isoetes*.<sup>1</sup>

It cannot be doubted that the outgrowth on the hypocotyledonary axis of *Gnetum* is equally the equivalent of these structures, though it owes its origin to an activity of growth of more deeply-seated tissues.

Looking at these several structures, when fully developed, from the purely histological point of view, we see that in the cases above cited the outgrowths are formed of tissues similar to those of the rest of the hypocotyledonary axis, and that they owe their origin to an increase of bulk, accompanied by cell division; but they have no special vascular system of their own.

Viewing the foot of the Fern in a similar manner we are led to a similar conclusion. If, in the case of the Fern, we

<sup>1</sup> Kienitz-Gerloff ('Bot. Zeit.,' 1881, p. 787) describes the origin of the cotyledon, root and foot in *Isoetes* from octants of the oosphere. From his account it appears that the foot is on the *same side* of the axis as the cotyledon; and in this his description coincides with the figures of Hofmeister. He then goes on to say that the orientation of these organs is exactly the same (*genau dieselbe*) as in the Filicineæ. In the Filicineæ, however, the foot is on the opposite side of the axis to the cotyledon.

consider the direction of the axis of the embryo as defined by the position of the apical cells of stem and root, the foot will here again appear as an outgrowth of tissues of the hypocotyledonary axis.

Thus we have a series of plants, starting with the Filicinae, in which structures, to be regarded histologically as outgrowths of the tissues of the hypocotyledonary axis (but which cannot be themselves included under the morphological terms of stem, leaf, or root), perform the physiological function of transfer of nutritive materials from the prothallus (or endosperm) to the growing embryo.

On comparing the orientation of these organs, *i.e.* their relation to the other members borne on the axis of the young plants, a want of uniformity will be found. In the Fern the foot is on the opposite side of the axis to the cotyledon. In Isoetes the foot is on the same side of the axis as the cotyledon. In Selaginella the foot is on the same side of the axis as the first formed cotyledon, though, since the two cotyledons appear almost simultaneously, too much stress must not be laid upon the point. In Welwitschia it has been shown that the feeder may appear on either side of the hypocotyledonary axis, the choice being determined according to the position of the seed during germination. In Gnetum the orientation of the outgrowth, relatively to the other members is not constant; the point at which it arises being also in this case determined according to the position of the seed during germination. A case, such as that of Gnetum, thus serves to throw light upon the want of uniformity of orientation in the Ferns and Ligulatae; as these outgrowths may vary in orientation in different plants of the same species, so also in various groups of plants the orientation of the outgrowth may vary according to varying conditions.

Further, we find that as we rise in the scale, the time of first appearance of these lateral outgrowths of the axis is successively later. In the Fern the foot is recognisable in very early stages of development of the embryo; from the constancy of its position, and of its origin in the true Ferns from two definite octants resulting from the first divisions of the oosphere, it has been regarded by some as being connected in some fundamental way with those octants.<sup>1</sup>

In Selaginella the foot first makes its appearance at a later stage of development of the embryo. In Welwitschia

<sup>1</sup> This view I held myself when writing my former paper on the germination of Welwitschia. For reasons which will be obvious from what is here written I have given up that opinion.

and *Gnetum* the formation of the outgrowth begins at a still later period, when the cotyledons, and apex of stem and root are already clearly defined.

This difference of time of first appearance of these outgrowths should be considered in its relation to the physiological position of the young embryo. In the Ferns the body of the embryo does not remain long embedded in the tissues of the prothallus; and the foot is thus required at an early period to transfer nutritive materials from the latter to the embryo, which is still unable to support itself. In *Selaginella* the embryo remains till a later period embedded in the tissues filling the macrospore, and the foot is accordingly required and developed at a later stage. In *Welwitschia* and *Gnetum* the embryo remains embedded in the endosperm till a still later period, and accordingly the outgrowth makes its appearance at a still later stage.

We have already seen that this lateral outgrowth of the hypocotyledonary axis is not a constant organ throughout the *Gnetaceæ*, it being absent, at least in some species of *Ephedra*; also that in *Welwitschia* and *Gnetum* it is proportionate in size and complexity to the size of the seed. These facts lead to the conclusion that in the *Gnetaceæ* at all events the outgrowth is formed only where it is required, and that its size and complexity varies according to the duties it has to perform. Referring to the vascular *Cryptogams*, we find a similar inconstancy of development of the foot. Cases occur in which the foot is not fully developed, and in which, were it not for the comparison of such embryos with the Ferns, the very slight lateral swelling of the axis would hardly be noticed. For instance, in *Equisetum* the foot always remains small, and is never fully developed (Sadlebeck, in Schenk's 'Handbuch der Botanik,' p. 224). In this genus, moreover, a distinct lateral outgrowth is not required, since the root of the young embryo perforates the prothallus, and the embryo remaining thus in close contact with it can draw supplies directly from it. A similar absence of a developed swelling of the hypocotyledonary axis is said to occur in *Azolla*.

In *Marsilea* we have a foot which is distinctly adapted in form to the function of transfer of nutritive matters; it differs from that of the true ferns in having a concave apex, which fits upon the convex surface of the prothallus.

We thus see that, in the vascular *Cryptogams*, as in the *Gnetaceæ*, these organs of nutrition of the young embryo appear on the hypocotyledonary axis as outgrowths of the tissue; they are not universally developed, nor are they,



throughout the vascular Cryptogams, of constant orientation relatively to the other members of the embryo.

Turning now to the Angiosperms, it has been shown by Tscherning,<sup>1</sup> Flahault,<sup>2</sup> Darwin,<sup>3</sup> Briosi,<sup>4</sup> &c., that in various plants belonging to different natural orders outgrowths of the peripheral tissues of the hypocotyledonary axis occur in the young seedlings. These outgrowths assume very different forms; their position is at the point of transition from stem to root; in most cases they are formed shortly after germination begins. In function these heels or pegs, as they have been called, differ from the organs which we have been considering. They have in no case been described as effecting a transfer of stored-up nutritive materials to the young seedling; their functions are chiefly mechanical. Looking at them from the point of view of structure, however, they coincide with the organs above considered, since they are formed by lateral extension of the tissues of the hypocotyledonary axis. The tissues outside the vascular bundles are chiefly concerned in their formation. These heels or pegs are found only in a comparatively small number of seedlings of the Angiosperms. It has already been observed that such outgrowths are not of universal occurrence in the vascular Cryptogams and Gymnosperms.

Drawing together the results hitherto obtained, we see that in a large number of vascular plants, from the Ferns upwards, the tissues of the hypocotyledonary axis are capable of a lateral extension, accompanied by division of the cells; that the outgrowths thus formed are usually seated at or near to the point of transition from stem to root; further, that the orientation of such structures is not uniform in all cases, nor, indeed, in some cases, even in the single individual (*e. g.* *Gnetum*, *Welwitschia*, *Cucumis*). Lastly, that, as we rise in the scale of development, the time of first appearance of the outgrowth is later, and may be seen to correspond to the time at which it will be required to perform its functions. The inconstancy of occurrence, orientation, and time of first appearance of these outgrowths seems to point to the conclusion that they are not to be regarded as clearly-defined morphological members, but rather as swellings of the tissue of the hypocotyledonary axis, which

<sup>1</sup> 'Unters. über die Entwicklung einiger Embryonen.' Inaug. Diss. Tübingen, 1872.

<sup>2</sup> 'Bulletin Soc. Bot. de France,' vol. xxiv, p. 201. 1877.

<sup>3</sup> 'The Movements of Plants,' p. 102. 1880.

<sup>4</sup> 'Sopra un Organo Finora non avvertito di alcuni Embryoni Vegetali.' Roma, 1882. Cf. also 'Bot. Zeit.,' p. 314. 1882.



arise only when and where they are required for the first processes of development and nutrition of the young embryo.

If now the term "foot" be applied to such outgrowths of tissue as appear on the hypocotyledonary axis (such as are found in the embryo of the vascular Cryptogams), and if the term be understood as not implying that the swelling holds any definite orientation relatively to the other parts of the embryo, or is connected essentially with any of the first divisions of the fertilised oosphere, the term "foot" might equally well be extended, so as to include all the cases of lateral outgrowth above enumerated, while it has this further advantage over the term "feeder," previously adopted for the lateral outgrowth in *Welwitschia*, that whereas in the majority of cases (vascular Cryptogams and Gnetaceæ) the function of the organ is chiefly one of nutrition; in the Angiosperms only a mechanical function has been ascribed to it, and the term "foot" does not imply any special function.

Returning now to the seedling of *Gnetum Gnemon*:

The arrangement of parts at the apex of the seedling is very similar in the three genera of the Gnetaceæ. There are found at a certain stage in each a pair of opposite cotyledons, a pair of plumular leaves decussating with these, a central apical cone, and a lateral bud in the axil of each of the cotyledons. In fact, in number and arrangement of parts the seedlings of the three genera are identical. From this point starts that divergence of character which makes the Gnetaceæ one of the most remarkable groups in the vegetable kingdom. In *Welwitschia* the further development of appendicular organs is arrested (with the exception of the adventitious fertile branches), while those already formed increase in size, or are thrown off (cotyledons). The internode between the cotyledons and plumular leaves remains in this case short. In *Ephedra* the above organs only attain a very limited size. At the apex are formed fresh decussating leaves with buds in their axils and elongated internodes. All the leaves with exception of the long linear cotyledons remain of a small size.

In *Gnetum* the development of fresh leaves proceeds as in *Ephedra*, but these have an expanded lamina and reticulate venation. Thus, from seedlings which are very closely similar are derived plants of most different habit.

The vascular system of *Gnetum Gnemon* differs from that of the other two genera, and the difference seems to depend to a great extent upon the fact that an uneven number of

vascular bundles enter the stem from the leaves of *Gnetum*, while from those of *Welwitschia* (at all events from the cotyledons and young plumular leaves) and *Ephedra* the number is two.

In the hypocotyledonary stem the bundle system resembles that of *Pinus pinea* ('Conif. und Gnet.,' p. 266-7), there being a comparatively large number of separate bundles, which do not rotate in the hypocotyledonary stem, while the bundle sheath does not appear till the point of transition from stem to root. At this point the xylem of only two of the bundles rotates on its axis, while the phloem of each of these divides into two parts, which mutually fuse to form the primary phloem of the root. The rest of the bundles pursue a direct course downwards, and are lost in the cambium of the root.

In the minute structure of the tissues the three genera are known to have many characters in common. In *Gnetum*, however, laticiferous tissues are present in considerable quantity; in this property it stands apart from the other members of the group.

Taking into consideration all the characters above described, the general result of this investigation is to show that the group of the Gnetaceæ is a still more natural one than it has hitherto been known to be: though the three genera do not correspond very closely to one another in the first cell divisions in the embryo, still the older seedlings show many points of remarkable similarity both in external form and internal structure. Further, it will be seen that *G. Gnemon* is in reality but little more closely comparable with the Angiosperms than the other two genera. In the development of its embryo it obviously follows the type of the Coniferæ, and indeed approaches them in some respects more nearly than either *Ephedra* or *Welwitschia*.

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*The ORGAN of JACOBSON in the DOG.* By E. KLEIN, M.D.,  
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IN my previous memoirs<sup>1</sup> the general arrangement and structure of the organ of Jacobson in the guinea-pig and rabbit, as well as the relations of the organ to the canals of Stenson, were minutely described, and in the present paper I propose to follow up the subject in the dog.

Exposing the septum nasale in its front part in the animal just killed we notice, close to the lower nasal furrow on each side, a small projection, running parallel with the nasal furrow, above it is another larger projection of the same direction; this latter curves upwards in the most anterior portion of the septum, exactly above the incisor teeth, while the former is at this region lost altogether. The upper larger projection is a solid fold of the mucous membrane of the nasal septum, while the lower one indicates the position of the organ of Jacobson.

On comparing transverse sections through this lower projection, which for brevity's sake we may call Jacobson's projection, with transverse sections for some distance in front of it, we perceive that the organ of Jacobson extends considerably further in front than is indicated by the above projection.

In the guinea-pig and rabbit the projection indicating the organ of Jacobson was due to the presence of the organ as a whole, but in the dog this is not the case, since Jacobson's projection is due partly to a peculiarity in the shape, and to a peculiar prominence of the cartilage of Jacobson, as will be pointed out in detail below, and not to the presence of the organ as a whole. In the front part of the organ this peculiarity of the cartilage is wanting, and the projection of Jacobson is absent, although the organ is still continued in

<sup>1</sup> This Journal, 1881, January, April and October.

this part beneath the superficial mucous membrane. In sections through the region of the posterior part of the organ it is seen that the organ as a whole may be already disappearing, while the projection of Jacobson is still marked, owing to the peculiar shape and the persistency of Jacobson's cartilage beyond the epithelial portion of the organ of Jacobson. Comparing figs. 8 and 10 this is well seen; fig. 8 is a representation of a section anterior to the projection of Jacobson, fig. 10, through the projection; in both the organ of Jacobson is well represented, but in fig. 10, *i.e.* the one through the projection, a peculiar prominence of the cartilage of Jacobson is noticed, to which, in part at any rate, the above projection is due.

Fig. 13, representing a section through the posterior portion of the organ, shows still the projection, although the organ of Jacobson is altogether shifted higher than the projection, and in a section a little behind the one represented, the epithelial part of Jacobson's organ has come to an end, the cartilage of Jacobson is still present, and the projection is well marked.

In a middle-sized dog the whole length of Jacobson's projection is between 2 and 3 centimeters. Anteriorly it loses itself gradually towards the nasal opening of the naso-palatine or Stenson's canal. About 1 centimeter behind the opening of this the projection takes its start.

In the guinea-pig and in the rabbit we have shown that the tube or organ of Jacobson opened with its narrow mouth directly into the nasal furrow (see my former papers, this Journal, January, 1882), and my friend the late Dr. Reuben Harvey has shown the same condition to obtain in the rat, hedgehog, and kitten. But in the dog I find the relations to be different, for here the tubes or organs of Jacobson do not open directly into the nasal furrow. Following in transverse sections the Stenson's canals, from their funnel-shaped opening into the anterior end of the nasal furrow down to their oral opening situated immediately behind the middle incisors and marked by a large papilla, about 0.5 centimeters in diameter, it will be seen that the tube or organ of Jacobson remains associated as a closed tube with Stenson's canal to near the oral opening of this latter, when it ultimately becomes fused with it.

Figs. 1 to 7 show the relation of Stenson's canal of one side to the organ of Jacobson of the same side, in transverse sections from the opening on the surface of the palate to the point at which the organ of Jacobson becomes separated from Stenson's canal. In these figs. at *pp* the Papilla is



shown with its thick stratified epithelium and its well-developed and long papillæ; at *o* is the opening of the canal on the surface of the palate; at *p* is the mucous membrane of the palate at the side of this opening; the epithelium is here much thinner and the papillæ shorter than on the Papilla. It is seen in figs. 1 and 2 that near the opening or mouth the Stenson's canal enlarges in a sort of sinus, and that it (*i.e.* the canal) is surrounded on its outer side by a trough-shaped plate of hyaline cartilage, indicated at *c* of the figures; this cartilage is Stenson's cartilage. As is seen in figs. 5 and 6, the upper part of Stenson's canal becomes gradually constricted off from the rest, and represents then the beginning of the organ of Jacobson, as in fig. 6. At the same time it is seen that Stenson's cartilage undergoes some change in position, the lower part of it disappears, whereas the upper part curves round the upper part of Stenson's canal; and when this becomes constricted off as the organ of Jacobson, the cartilage forms a curved plate round this latter, so that while the cartilage was previously situated on, and curved round the outer surface of Stenson's canal, it is now situated on, and curved round the median or inner surface of the organ, and consequently is now no more Stenson's but Jacobson's cartilage. Compare figs. 3, 4, 5, and 6. This separation of the organ of Jacobson from the Stenson's canal, as indicated in fig. 6, occurs still on the oral side of the osseous palate; immediately before, and while traversing this latter the canal of Stenson and the tubes or organs of Jacobson retain exactly the same relative position. And also the cartilage retains its shape and position, *i.e.* being a curved cartilage plate on the median or inner side of Jacobson's organ (see fig. 7), *i.e.* Jacobson's cartilage.

The structure of the different parts is as follows:

1. The canal of Stenson. (*a*) The epithelium lining the canal is throughout stratified. The stratified pavement epithelium of the palate, together with its superficial stratum corneum, is continued into the mouth of the canal, but there are no papillæ in this part, *i.e.* the mouth of the canal. Very soon, however, when the canal enlarges, *i.e.* above the mouth, the stratum corneum of the surface is lost, and the epithelium, although remaining stratified, nevertheless changes its character somewhat, inasmuch as its superficial cells are not much flattened, but are distinctly polyhedral or even cubical cells; the cells of the middle layers are polyhedral, and those of the deep layers are much elongated in a vertical direction, cylindrical or spindle shaped. The

epithelium remains of this character throughout the canal. (b) Underneath the epithelium is the mucous membrane, which, like that of the palate, is dense fibrous tissue with the usual capillary networks. Numerous elastic fibrils running in a longitudinal direction are met with between the bundles of connective tissue. There are indications only of papillæ. The tissue belonging to the wall of the canal is not well defined from the surrounding fibrous tissue, except that it is loose in its texture. (c) The cartilage is hyaline and does not present any peculiarity of structure. The shape of it and its relation to Jacobson's cartilage have been mentioned above.

The following measurements give an idea of the size of these different parts :

At a point illustrated in fig. 6 the long transverse diameter is about 1.17 mm. The short transverse diameter through the broadest point about 0.675 mm. The thickness of the epithelium is about 0.3375 mm.

2. The organ of Jacobson. As has been pointed out above, the organ or tube of Jacobson opens into Stenson's canal below the bone of the palate ; from this opening to the point where the organ of Jacobson has entered the nasal septum, *i.e.* beyond the point where the Stenson's canal has opened into the nasal furrow, and consequently has ceased to exist as such, the organ of Jacobson is comparatively a small tube with a lumen circular in transverse section.

Figs. 6, 7, and 8 show these points distinctly. In figs. 6 and 7 the organ of Jacobson is still in company with Stenson's canal and below the osseous palate ; in fig. 8 Stenson's canal is no more, and the organ of Jacobson is already contained in the nasal septum.

Throughout the rest of the organ except the most hind portion, the lumen of the organ is more or less kidney-shaped, owing to the lateral wall possessing a convex, the median wall a concave, surface. In about the middle of the organ and a little anterior to it, this condition is best developed. In figs. 9, 10, and 11 the relative size and shape of the organ are well shown. At the posterior extremity of the organ the transverse diameter decreases considerably, and the shape of the organ alters in this manner, that although its lumen in transverse section is still convex-concave, the upper sulcus of it extends upwards in the shape of a long thin cleft. At the very end of the organ the lumen becomes smaller, elliptical in transverse section, and ultimately is altogether lost. In figs. 12 and 13 these relations are easily recognised.

Before entering into the description of the minute structure of the organ, I wish to point out the relations of the osseous support of the organ in the nasal septum.

Immediately before passing through the bone of the superior maxilla, *i.e.* immediately behind the incisor teeth, it is noticed that the osseous bit next the median line and joined here by periosteum, forms a direct continuity with the rest of the maxilla, see fig. 7. These median bits of bone, representing the intermaxillary bone, extend backwards as the crista nasalis, and are separated from the rest of the jaw by thick layers of fibrous tissue.

From the point where the organ of Jacobson has entered the nasal septum (fig. 8) to the hind extremity of the organ it is supported on its median and lower part by the bone of the crista nasalis (intermaxillary bone). As is shown in figs. 8, 9, and 10, which are taken from the anterior third of the organ, the organ of Jacobson is supported on its median aspect by the spongy bone of the crista nasalis, these bones being joined in the middle line by a thin layer of periosteum. But in the middle and hind portions of the organ this osseous support is greatly increased by a plate of spongy bone above, and by its becoming again joined to the palatine plate of the jaw. In fig. 11 these relations are easily understood; 2 is the spongy bone which is a continuation of the osseous support shown in figs. 8, 9, and 10; 1, however, is a new addition, and 3 is the palatine plate of the jaw. All these various bits of bone are spongy in structure, and are joined to one another and to their companion on the opposite side by a thin layer of vascular periosteum.

From this description and from a comparison of the figures given in the present memoir with those representing the organ of Jacobson in the rabbit and guinea-pig given in my previous papers, it is seen that the relation of the organ of Jacobson to the crista nasalis or the intermaxillary bone respectively, is quite different in the three animals. Compare this Journal, January, 1881, Plate VII, April, Plates XVI and XVII, October, Plates XXX and XXXI.

In the description of the organ of Jacobson we shall here follow the same plan as adopted in my previous memoirs, *viz.* considering first the cartilage of Jacobson, then passing on the lateral, and then to the median wall of the organ. Under lateral wall we shall also here understand the wall nearest to the outer surface of the nasal septum, whilst the



median wall is the one nearest to the middle line of the nose.

(a) The cartilage of Jacobson. As has been described above, at the most anterior section of the organ the cartilage of Jacobson is directly continuous with that of Stenson's canal (figs. 4 and 5). At first, and before entering the nasal septum, the cartilage of Jacobson is a curved plate, crescentic in transverse section (figs. 5, 6, and 7), and increasing in thickness and extent from forward to backward. Having entered the nasal septum the cartilage of Jacobson, while considerably larger than in front, has become crook-shaped, as is shown in fig. 8. We may now distinguish on it a larger or vertical labium and a smaller or horizontal labium. The former is much thicker at its upper extremity than in any other part. Passing backwards, we find in the whole length of the organ the cartilage of the same shape and position as is seen in figs. 9, 10, and 11, but the size varies, for the vertical labium becomes very much longer than in front (see figs. 10 and 11). In the middle part of the organ the horizontal labium of the cartilage possesses a minute lateral projection (fig. 10), which causes here a similar bulging out of the mucous membrane of the surface of the nasal septum—Jacobson's projection. Near the posterior extremity Jacobson's cartilage changes slightly in shape, as the extremity of the horizontal labium becomes slightly curved upwards (see fig. 12). At the very end of the organ the horizontal labium altogether disappears, and also the vertical labium becomes slightly shortened (fig. 13). This horizontal labium extends for a short distance beyond the extremity of the organ of Jacobson as a smooth plate of cartilage, and ultimately terminates with a blunt extremity.

As regards the structure of Jacobson's cartilage there is very little to be said; it is hyaline cartilage, many of the cartilage cells containing either one large or many small oil globules. In the latter instance the cells show a beautiful honeycombed network as a framework for the oil globules.

Before the organ of Jacobson enters the nasal septum, *i. e.* before it passes through the bone, and while still contained in the membrane of the palate, the epithelium lining the lumen is stratified pavement epithelium of very nearly the same nature and thickness as that lining Stenson's canal. Underneath the epithelium is a dense fibrous membrane, containing in the lateral wall, *i. e.* the wall next the canal of Stenson (see figs. 6 and 7), very numerous lymphoid corpuscles. Outside the subepithelial dense membrane is a



loose fibrous texture containing numerous large blood-vessels, arteries, and especially veins, running parallel with the long axis of the organ. In an upward and downward direction we meet with small short glands, opening with their ducts into the lumen of Jacobson's organ. The glands are composed of a few acini, and these are in structure serous glands of exactly the same nature as those described of Jacobson's organ in the guinea-pig and rabbit.

In the median wall I can trace here and there a branch of olfactory nerve-fibres. The organ having entered the nasal septum is at first lined with the same stratified pavement epithelium as before, about 0.0864 mm. in thickness; the subepithelial membrane is much infiltrated with lymphoid cells, especially in its lower part. In the median wall we find amongst the numerous large blood-vessels a few branches of olfactory nerves running in a longitudinal direction. The serous glands are met with in the same position as before. We now find a distinct layer of fibrous bands, by which the lateral wall of Jacobson's organ becomes differentiated from the mucous membrane lining the surface of the nasal septum in this region. If we imagine in fig. 8 fibrous bands of the perichondrium of Jacobson's cartilage passing from the lateral aspect of the extremity of the ascending to the lateral aspect of the extremity of the horizontal labium, we have a correct idea of the position of these structures. So that these bands separating the lateral wall of Jacobson's organ from the outside membrane would have the position of a chorda to the arch represented by the hook-shaped cartilage of Jacobson (see fig. 8).

And throughout the rest of Jacobson's organ its lateral wall is bounded on its outside in this same manner (see figs. 9, 10, 14, and 15).

But very soon after its entrance into the nasal septum the epithelium of the organ of Jacobson changes its character. As long as its lumen is circular in transverse section its epithelium is stratified pavement epithelium, but the lumen becoming triangular, and then oblong, the epithelium changes into columnar epithelium.

This change does not take place simultaneously in the two sides, for while in the organ of one side the whole lining epithelium may be found to be columnar, that of the organ of the other side is still stratified pavement epithelium in the upper and lower part of the wall.

As soon as the lumen of the organ in transverse section is kidney shaped the epithelium all round is columnar; that of the median wall is different from that lining the lateral

wall. Just as was the case in the Jacobson's organ of the guinea-pig and rabbit, so also in the dog, the epithelium lining the median wall is supplied with olfactory nerve-branches, and contains their terminations in the shape of peculiar large cells. We shall therefore consider the epithelium lining the median wall as the sensory epithelium. The shape of the lumen of the organ in transverse section being oval, we shall call the upper part the upper sulcus, the lower part the lower sulcus. The whole extent of the lateral wall of the organ including the upper and lower sulcus is distinct and different in structure from the rest of the wall of the organ. The former will be understood to represent the lateral wall in its broader sense, the latter the median wall proper.

I. The lateral wall. The epithelium lining this wall on the inner free surface is columnar epithelium of exactly the same nature and thickness as the one covering the surface of the mucous membrane of the nasal septum and the nasal furrow, viz. it is composed of three different kinds of cells: (*a*) a superficial layer of conical or cylindrical cells, with a bundle of fine cilia on the free surface; then (*b*) a layer of spindle-shaped cells pushed in between the extremities of the first-named cells; and (*c*) a deep layer of cells more or less conical, but with their base resting on the subepithelial membrane. In some sections I have met with goblet cells amongst the superficial ciliated epithelial cells. The thickness of the epithelium is about 0.048 to 0.072 mm., exclusive of the cilia, which are about 0.006 mm. in length.

Underneath the epithelium is a dense fibrous membrane, containing numerous lymphoid corpuscles; this infiltration with lymph-cells is more pronounced in the most anterior portions of the organ than further behind.

A large number of fine elastic fibrils, running longitudinally, are met with in the subepithelial membrane. Partly within this, but especially between it and the fibrous bands mentioned above as separating the lateral wall from the mucous membrane of the nasal septum, are numerous vessels, most of them running parallel with the long axis of the organ. Some of these are arteries, but the majority are venous vessels, and they form a plexus. There appears in this respect no difference between the lateral wall of the organ and the mucous membrane covering the free surface of the nasal septum, which is also richly supplied with a plexus of small veins.

All glands of the organ of Jacobson are serous glands, of exactly the same nature as in the organ of the guinea-pig

and the rabbit, and also the mucous membrane of the nasal septum is richly supplied with the same serous gland.

The glands are small, and they are present only in small numbers about the region of the upper and lower sulcus; in the former they are larger and more numerous than in the latter. Their ducts open into the upper or lower sulcus respectively; the lining epithelium is a single layer of columnar cells without cilia, but continuous with the epithelium of the lumen of the organ.

The glands of the upper sulcus can be traced to form a continuity with the glands of the mucous membrane of the nasal septum.

Only exceptionally are there any glands present in the middle of the lateral wall, and in this case their duct opens straight in the middle of the surface of the lateral wall. This condition obtains especially in the hind portion of the organ, but is only rarely met with in the anterior part.

The difference in structure of the lateral wall of Jacobson's organ in the rabbit and dog are then considerable, as will be easily seen from a reference to my paper in the October number, 1881, of this Journal. Besides the difference in the shape of Jacobson's cartilage in the two cases, and besides the difference in the amount of the serous glands discharging their secretion into the cavity of the organ of Jacobson, there is this striking difference between the two cases, that in the dog the cavernous tissue, so strongly developed in the lateral wall of the organ in the rabbit and also in the guinea-pig, is altogether absent from the lateral wall of the organ in the dog.

II. The median wall. (a) The sensory epithelium is a conspicuous part of the median wall. As is the case in the organ of the guinea-pig and rabbit, so also in the dog, the sensory epithelium is well defined from the epithelium of the lateral wall and that lining the upper and lower sulcus. Besides, the sensory epithelium differs in structure from the rest of the epithelium, for here we find the following elements:—(a) A superficial layer of very thin long conical, or spindle-shaped, or cylindrical *epithelial* cells, each with an oval nucleus; no cilia are to be seen on the surface; the cells are longer and thinner than the ciliated cells of the superficial layer of the epithelium of the lateral wall. The individual *epithelial* cells are possessed of a single or divided or branched process, extending into the deeper layers of the sensory epithelium; the opposite extremity, *i. e.* the one reaching the free surface of the epithelium, is either thin and rod-shaped or it spreads out fan-like, and with the free



margin of this enlargement forms part of the general surface. There is generally noticeable on the free surface a cuticular, sharply-defined boundary layer, similar to the outer limiting membrane of v. Brun of the olfactory organ. The same layer was described also in the organ of the rabbit. The nuclei of the superficial *epithelial* cells of the sensory epithelium are not disposed in a single plane, but the individual epithelial cells possessing their nucleus at variable heights it follows that, viewing the layer of *epithelial* cells as a whole, we find their nuclei disposed in two or three planes. In a vertical section through the epithelium, such as is represented in fig. 15, this point is well shown. In such sections stained with logwood or anilin dyes the contrast between these *epithelial* cells and the cells below them is very marked, just as was the case in the organ of the rabbit; the *epithelial* cells, by their deeply-stained nuclei, differ conspicuously from the layer of clear nuclei beneath. ( $\beta$ ) Underneath this stratum of *epithelial* cells is a stratum of *sensory* cells. In the guinea-pig and rabbit I have shown that this stratum consists of several layers of sensory cells, but in the dog there exists, as a rule, only a single, or at most in some places a double, layer of these cells. In fig. 16 these cells are accurately represented; they appear large ganglionic cells, each possessing a clear, relatively large, more or less spherical nucleus. Each of these nuclei contains, within a definite limiting membrane, a well-developed uniform reticulum with one or two nucleoli.

The substance of these sensory cells is chiefly accumulated on the side of the nucleus nearest to the epithelial cells, *i. e.* directed toward the free surface, and it extends either in the shape of a single or divided thinner or thicker process up to the free surface. This is the outer process; but on the opposite pole there is also one or more processes directed downwards, *i. e.* towards the depth; these are the inner processes. They are much finer, and, as is obvious from the inspection of fig. 15, much shorter than the outer processes.

( $\gamma$ ) Underneath these *sensory* cells is a limiting layer of small cells, which in specimens hardened in chromic acid appear more or less branched and connected with one another; their nuclei are spherical and smaller than the nuclei of the sensory cells; some of these deep cells appear conical, possessing a basis with which they rest on the sub-epithelial membrane, and their apex process is directed in the opposite direction between the sensory and superficial epithelial cells. From this it must be clear that this deep



boundary layer of cells corresponds to the similar layer of inverted conical cells present in the epithelium of the lateral wall, and in the epithelium of the olfactory region in some instances.

On the free surface of the sensory epithelium we notice in sections of hardened specimens numerous fine hair-like projections which are, however, very different from the cilia of the epithelial cells of the lateral epithelium, inasmuch as they are not so long and not so definite hairs as the cilia, but rather represent in their totality an indistinctly striated substance. Whether these striæ are prolongations of certain cells only, *e.g.* the outer processes of the *sensory* cells, or whether they are merely prolongations of all cells indiscriminately, *i.e.* *epithelial* cells and *sensory* cells, due to the action of the reagents used, I have not been able definitely to ascertain.

As regards the extent of the sensory epithelium in the median wall, it differs slightly in the two sides at the same point of the nasal septum, but especially is this the case in different places of the same side. The following measurements illustrate this:

In a transverse section, such as is represented in fig. 9, the extent of the sensory epithelium in the median wall is about 0.32 mm., about one fifth of the whole circumference.

In a section such as is represented in fig. 11 the extent of the sensory epithelium on one side is 0.72 mm., of that of the other side 0.62 mm.

In a section represented in fig. 12 the extent of the sensory epithelium is about 0.5, in fig. 13 about 0.38 mm. As a rule, the extent of the sensory epithelium in the median wall is greatest in the middle parts of the organ, amounting to about one third of the whole circumference; it diminishes anteriorly and posteriorly.

The thickness of the sensory epithelium varies between 0.0576 mm. and 0.1 mm.

(b) Underneath the sensory epithelium is a fibrous membrane containing in some few places lymphoid cells. As a rule there are here numerous venous vessels running longitudinally, as well as branches of olfactory nerves; these form a plexus under the epithelium, and ultimately ascend in an oblique or vertical manner into the sensory epithelium. In fig. 17 these ascending branches are well seen.

The most important points in connection with the median wall is naturally the presence of numerous branches of olfactory nerves, and their relation to the sensory epithelium. In these respects I have found exactly the same con-

ditions to obtain as I described of the organ in the rabbit. The minute nerve branches mentioned above as ascending into the sensory epithelium become connected by their fibrils with the inner processes of the sensory cells (see fig. 12 of Plate XXXI, in the October number, 1881, of this Journal).

Nearer to the cartilage of Jacobson, and in an upward direction, we meet with some larger vessels running longitudinally and surrounded by bundles of non-striped muscle, and these vessels appear to me to be the only thing that can be interpreted as a rudiment of a cavernous tissue, such as is present in the lateral wall of the organ in the guinea-pig and rabbit.

The large branches of the olfactory nerve running longitudinally are also met with in this outer layer of loose tissue. Numerous fine elastic fibrils running parallel with the long axis of the organ are met with in all parts of the median wall.

In the upper portion of the median wall a few serous glands are occasionally seen, but they and their ducts really belong to the region of the upper sulcus, as is well seen in fig. 15.

In sections of hardened preparations there is a finely granular precipitate present on the surface of the sensory epithelium; this is probably coagulated secretion of the gland that had been poured into the cavity of the organ.

*On SAPROLEGNIA in RELATION to the SALMON DISEASE.*

Extracted from the Twenty-first Annual Report of  
H.M.'s Inspectors of Salmon Fisheries.

In willingly assenting to the proposal of the Editor of the 'Quarterly Journal of Microscopical Science' that the following extract from Mr. Walpole's and my Report should appear in the Journal, I adopt their suggestion that I should take upon myself all responsibility for what is there written after the words, "The first symptoms of this disease," on p. 312.

T. H. HUXLEY.

JUNE 23, 1882.

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THERE are two other matters on which it is necessary for us to treat before we close this report; one of these we need only refer to in a short paragraph; the other we propose to deal with in more detail.

In the first place, it is our duty to point out that the multiplication of salmon is seriously affected by the increase of pollution. Into the particulars of these pollutions it is not necessary for us to enter. A reference to the Appendix will show the many cases in which new pollutions have arisen, or old sources of pollution have done fresh damage during the last twelve months, and the few cases in which steps have been taken to render pollution harmless. We may say generally that the multiplication of fish is made more difficult in this way, and that some of the rivers which have hitherto been most productive are in perhaps the greatest danger from this cause. It is for Her Majesty's Government, rather than for ourselves, to consider whether, under these circumstances, any steps are desirable for the purpose of remedying these pollutions. It is merely our duty to point out the danger which arises from them.

The second point to which we desire to draw attention is the remarkable outbreak of a disease among the salmon of

many rivers. The disease was noticed originally in the autumn and spring of 1877 in two rivers, the Esk and the Nith, which flow into the Solway Firth. It soon spread to the Eden and other adjoining rivers. In the spring of 1879 it was observed in the Tweed, when it rapidly became very serious, and in 1880, when a commission was appointed to investigate it, it had extended to the Nith, the Annan, the Esk, the Eden, the Cree, and the Dee, all flowing into the Solway Firth; to the Doon and the Ayr in Ayrshire; to the Derwent in Cumberland, the Lune in Lancashire, and to the Tweed. Since then the disease has broken out in the Seiont, the Ogwen, and the Conway in North Wales, and in the Tay and North Esk in Scotland.

We have very little doubt that the disease which first excited attention in 1877 had existed, at any rate in a sporadic form, for many years. It was stated in evidence before the late commission that Dr. Crosbie, formerly surgeon to the "Challenger" expedition, carefully investigated a case of the disease so long ago as in 1852. His observations will be found in the Commissioners' Report, p. 44. Other witnesses similarly stated to the commissioners that they had observed sporadic cases of the disease for years. We may add that we have recently understood from a lessee of fisheries on the North Esk that he had seen diseased fish, without recognising them as diseased, for very many years; and we have very little doubt that sporadic cases of the disease occur in almost every river.

The first symptom of this disease is the appearance of small greyish or ashy discolorations of the skin, usually upon those parts of the body which are devoid of scales, such as the top and sides of the head, the delicate valvular membrane on the inside of the jaws, the adipose fin, and the soft skin at the bases of the other fins. Where such discoloured patches occur on the scaly parts of the body the scales are hidden by a film, and it might readily be supposed that they had been detached. But if the discoloured film is gently washed or wiped off, the scales will be found beneath perfectly undisturbed. On the scaleless part of the body, also, the discoloured places often look as if they were the effect of bruises or abrasions, but careful examination of the skin fails to reveal any evidence of external injury.

The exact character of this affection of the skin may best be observed in the recently formed isolated patches, not bigger than a sixpence, in which the disease appears on the soft integument of the head. Such a patch is usually nearly circular and has a well-defined margin separating it from



the healthy skin. The central region of the patch is somewhat raised and more discoloured than the rest; and faint ridges may commonly be seen radiating from it, through the marginal zone, to the edge of the patch. A single patch of this character may be observed on a fresh-run fish, which from its activity, the excellent condition of its flesh, and the perfectly normal aspect of its internal organs, shows itself otherwise to be in full health.

When a patch of diseased skin has once appeared, it rapidly increases in size and runs into any other patches which may have appeared in its neighbourhood. The marginal zone, constantly extending into the healthy surrounding skin, retains its previous characters, while the ashy central part changes. It assumes the consistency of wet paper, and can be detached in flakes, like a slough, from the skin which it covers. If the subjacent surface is now examined, it will be found that the *epidermis*, or scarf-skin, has disappeared, and that the surface of the vascular and sensitive *derma*, or true skin, beneath is exposed. As the diseased area extends the papyraceous coat more and more completely takes the place of the epidermis, until, in extremely bad cases, it may invest the back and sides of a large salmon from snout to tail.

The affection, however, is not confined to the epidermis. As the patch acquires larger and larger dimensions the derma, or true skin, in its centre becomes subject to a process of ulceration; and thus a deep bleeding sore is formed, which eats down to the bones of the head and sends off burrowing passages, or sinuses, from its margins.

In severe cases the skin of the top of the head, of the snout, of the gill covers, and of the lower jaw, may be almost completely destroyed, and the affection may extend far into the interior of the mouth. Cases of the blinding of fish by extension of the disease over the eyes are reported. It is also said that the gills are attacked; but, although careful attention has been paid to this point, the gills have been unaffected in every fish that has come under our notice, however severe and extensive the disease might be. In far advanced cases the edges of the fins become ragged; and sometimes the skin which invests the fin rays is so completely destroyed that they stand out separately.

All observers agree that the flesh of a diseased salmon, however extensive the morbid affection may be, presents no difference in texture or in colour from that of a healthy fish; and those who have made the experiment declare that the flavour of a diseased fish is as good as that of a healthy one.

No morbid appearances are discoverable either in the viscera or in the blood. Moreover, when fresh-run fish are diseased they may exhibit just as large an accumulation of peritoneal fat as healthy fish. Nevertheless, it is certain that the cutaneous affection causes much irritation. The fish exhibit signs of great uneasiness, often dashing about and rubbing themselves against stones and other hard bodies in the water. Eventually they get weaker, become sluggish, and often seek the shallows before they die.

The disease spreads with great rapidity after it has commenced, three or four days being said to be sufficient to enable it to extend over the whole body of a large salmon.

In the early stages of the malady the peculiar appearance of the parts of the skin affected might readily be, and certainly often has been, ascribed to mechanical injury. It has already been remarked that the scales often appear to have been detached when in reality they are only hidden by the pellicle which covers them; nor, so far as inspection with the naked eye goes, is there anything to suggest that the disease, in its most advanced form, is anything but a sloughing ulceration of the skin. But when the papyraceous substance which constitutes the apparent slough is subjected to microscopic examination, it proves to be something totally different from mere dead tissue of the fish, such as a true slough would be. In fact, the comparison with wet paper turns out to be more exactly correct than might have been anticipated; for, like wet paper, it is chiefly composed of a felted mass of vegetable filaments, intermixed with which are *débris* of the tissues of the skin of the salmon and all sorts of accidental impurities, especially shells of Diatoms and multitudes of very minute sand grains, derived from the water in which the salmon swim. The filaments vary in thickness from  $\frac{1}{600}$  of an inch to  $\frac{1}{3000}$  of an inch, the majority lying between  $\frac{1}{3000}$  and  $\frac{1}{2000}$  of an inch. Each filament is tubular, composed of a thin wall, which contains cellulose, or the essential proximate principle of wood, lined by a thicker or thinner layer of finely granular protoplasm, within which, again, is a watery fluid. The whole filament is colourless and usually transparent, but sometimes the granules are sufficiently numerous to render it opaque, and then it looks white by reflected light. Sometimes the filaments are simple as far as they can be traced; sometimes, on the other hand, they are much branched; but they never exhibit any transverse partitions, the cavity of each filament being continuous throughout. Wherever the free end of a

filament is to be seen it is rounded, closed, and often no larger than the rest; or the filament may taper to its extremity. But the free ends of a greater or less number of the filaments are slightly enlarged, so as to be club-shaped, or they may be pyriform, or even almost spheroidal, and the layer of protoplasm which they contain is very thick. The cavities of some of these enlarged ends are shut off by a transverse partition from the rest of the filament, thus giving rise to a closed case. In others the protoplasm is broken up into a number, greater or less, according to the size of the enlargement, of equal sized spherical masses, each rather less than  $\frac{1}{2000}$  of an inch in diameter, which lie separate, but closely packed in the interior of the case, like shot in a cartridge (Fig. I, p. 316). In others the case is seen to be open at the end, and a portion or the whole of the "shot" have passed out. In yet others, again, a full unopened case is seen to lie inside an empty one.

The papyraceous mass is, in fact, what is known as the *mycelium* of a fungus. It answers exactly to the similar, wet-paper like crust which is formed by the common fungus, *Penicillium glaucum* (usually known as "blue mould"), on the surface of a pot of jam. The filaments are the stems of the fungus, and are technically known as *hyphæ*. The enlarged ends of the hyphæ which are converted into the "cases," are the *sporangia*, or fruits of the fungus; and they are termed *zoosporangia*, inasmuch as the spheroidal bodies or *spores*, under certain circumstances, are actively locomotive after the fashion of many animalcules, and are, therefore, termed *zoospores*. It is a peculiarity of this particular fungus that, when a zoosporangium has emptied itself, the hypha on which it is supported begins to grow afresh, sends a prolongation through the centre of the empty sporangium, and dilates into a new one within or beyond it. Hence the appearance of a full sporangium, surrounded by one, or it may be two or three empty ones, one inside the other (Fig. II, p. 316).

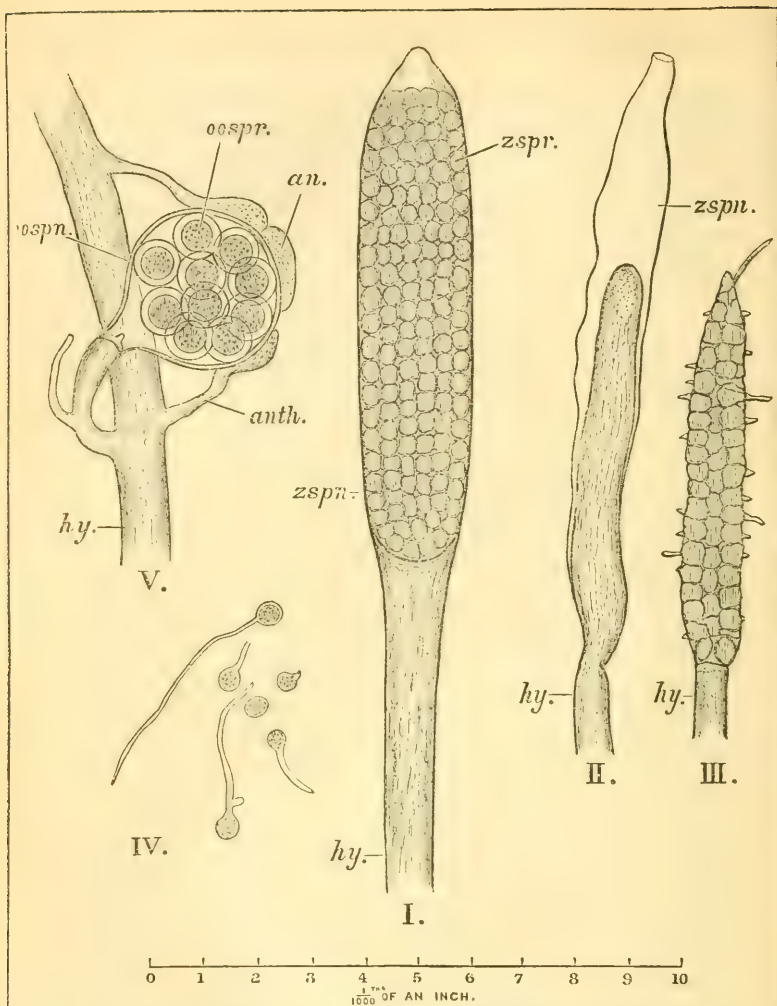
This structural feature is peculiar to the genus *Saprolegnia* among fungi, and it enables mycologists to identify the fungus, of which the papyraceous incrustation characteristic of the salmon disease is a product, as a species of that genus.

Thanks especially to the labours of Pringsheim,<sup>1</sup> Cornu,<sup>2</sup>

<sup>1</sup> "Die Entwickelungs-geschichte der Achlya prolifera." Nova Acta, 1851, and several later papers in the 'Jahrbücher für Wissenschaftliche Botanik' for 1857, 1860, and 1874.

<sup>2</sup> 'Monographie Annales des Sciences Naturelles, Botanique,' 1872.





*Characteristic Forms of the Sporangia and Spores of Saprolegnia.*

I.—A zoosporangium full of nearly ripe zoospores from the skin of a living diseased salmon.

II.—An empty zoosporangium, through the centre of which the hypha is growing in order to produce a new zoosporangium. From the fresh growth of *Saprolegnia* on the diseased jaw membrane of a salmon, cut off and placed in water.

III.—A dictyosporangium from salmon *Saprolegnia* cultivated on a dead fly. The spores have remained in the interior of the zoosporangium, and, after encasing themselves, have there germinated.

IV.—Zoospores of salmon *Saprolegnia*, germinating in water.

V.—An oosporangium of *Saprolegnia* from the pike, cultivated on a dead fly. The oosporangia of the salmon fungus in all respects resemble this.

Signification of the letters:—*hy*, hypha; *zspn*, zoosporangium; *zspr*, zoospore; *oospr*, oosporangium; *oospn*, oospore; *anth*, antheridial filament; *an*, antheridium.



De Bary,<sup>1</sup> and Brefeld,<sup>2</sup> a great amount of accurate information respecting the *Saprolegnia* has been accumulated of late years.

They may be defined as a kind of water-moulds, which usually live at the expense of dead and submerged animal and vegetable substances, and are especially common upon dead insects and other invertebrate animals. Their delicate hyphæ form a white cottony fringe to such matters.<sup>3</sup>

A dead fly which has fallen into water is a favourite nidus for *Saprolegnia*, the hyphæ of which radiate from it in all directions, so that the fly appears to be enclosed in a pale white fluffy ball. Careful examination shows that such a fly represents the soil in which an immense number of the minute *Saprolegnia* are implanted. One half of each fungus consists of branching hyphæ which answer, in a fashion, to the stem and branches of an ordinary plant, and are visible externally; the other half of the fungus corresponds, in the same general way, to the root and rootlets, the hyphæ ramifying in the interior of the fly, and the two parts being connected by a portion which traverses the dense cuticle with which a fly's body is coated.

The stem-hyphæ answer exactly in size and structure to the hyphæ of the salmon fungus. Moreover, a large number of them terminate in zoosporangia of the same character, which evacuate their zoospores, and are reproduced in the same way.

Flies, or parts of flies, such as the legs, on which *Saprolegnia* are healthily growing, can be isolated and watched for any needful time under the microscope, so that the whole process of the formation of the zoosporangia and zoospores can be followed step by step. It may then be observed that the simple subcylindrical free end of a hypha enlarges, that protoplasm accumulates in it, and that its cavity, finally, becomes shut off by a transverse partition from the rest of the hypha, as a zoosporangium, the summit of which is usually slightly conical. The protoplasm is then seen to break up, simultaneously, into from eight or ten to a hundred and fifty zoospores, according to the size of the zoosporangium. The apex of the latter then opens and the zoosporangia are emitted. Each zoospore, as it leaves the zoosporangium, is usually in active motion, being propelled by the rapid lashing of two vibratile cilia which are attached

<sup>1</sup> De Bary and Woronin. "Untersuchungen über die Peronosporéen und Saprolegnien," 1881.

<sup>2</sup> 'Botanische Untersuchungen,' Heft iv, 1881, p. 109, 110.

<sup>3</sup> Whence the name *σαπρός*, *sapros*, rotten, and *λέγνον*, *legnon*, the edging of a garment.

to one point of its surface. After a few minutes it becomes quiescent and surrounds itself with an extremely delicate transparent coat. But this repose is of a very short duration, as it soon emerges from its envelope, and moves about even more actively than before. It has now an elongated oval shape, and has two cilia which proceed from one side of the oval. This second active state may last for a day, or perhaps two; and it is obvious that, from the activity of the motion of the zoospores, to say nothing of accidental currents, they may thus be carried a long way from the parent stock. Sooner or later, however, they again come to a state of rest, which is final, and they then usually germinate. That is to say, one, or perhaps two, delicate filaments grow out and represent the primitive hyphæ of a new *Saprolegnia* (Fig. IV, p. 316).

If the spore has attached itself to some body which is incapable of affording it nourishment, it may not germinate at all, or, if it germinates, it speedily dies. But, if it falls upon an appropriate soil, such, for example, as the body of another dead fly, the spore sends a prolongation inwards which perforates the tough chitinous cuticle of the fly, and gives rise to a system of root-hyphæ in its interior; while, simultaneously, it grows outwards into a similarly ramifying stem-hypha, the branches of which soon enlarge into zoosporangia and give rise to zoospores, as before.

The growth and development of the *Saprolegnia* take place with extraordinary rapidity. In thirty-six hours from the first infection of the body of a dead fly with the *Saprolegnia* spores, it may be covered with a thick coat of stem-hyphæ a fifth of an inch long; and, in the course of the second or third day, a thousand of these may have developed and emptied their sporangia, thus setting free some 20,000 zoospores, every one of which is competent to set up the same process in a new fly-corpse. As all this production takes place at the expense of the tissue of the fly, the supply of nutritive material gradually diminishes. At about the fourth day, or perhaps not till later, new forms of sporangia, termed "dictyosporangia" (Fig. III, p. 316), in which the spores encase themselves and often germinate while still within the sporangium, make their appearance; and the ordinary zoosporangia diminish in number. Not unfrequently, about this time or subsequently, the hyphæ tend to break up into short joints which are themselves capable of germination. Finally, after the fifth or sixth day, a new kind of sporangium usually makes its appearance, which is termed an *Oosporangium*, inasmuch as the spores to which it gives rise

are more like eggs, or seeds, than the products of the zoosporangia or those of the dictyosporangia.

The summit of a hypha, or a short branch of a hypha, dilates into a spheroidal sac, the cellulose wall of which becomes thickened, but presents, here and there, thin places, looking like clear circular dots, or apertures, under the microscope. Protoplasm accumulates in the spheroidal case thus formed; and either remains a single rounded mass, or divides into a smaller or greater number of spheroids, each of which, much larger than a single zoospore, is an *Oospore*. The oospore, or oospores, thus formed eventually become invested by a thick cellulose coat. Before this happens, in some forms of *Saprolegnia*, slender twig-like branches are given off either from the stalk of the oosporangium or from an adjacent hypha, and the terminal portion of one or more of these twigs applies itself to the oosporangium. This terminal portion becomes shut off from the rest of the twig by a transverse septum, and is an *Antheridium*. The antheridium pierces the wall of the oosporangium, divides into as many branchlets as there are oospores, and one branchlet applies itself to each oospore. In all probability something passes from the antheridium into the oospore, and effects fecundation (Fig. V, p. 316).

Thus the oosporangium represents a female reproductive organ, and the oospore takes the place of an egg or an embryo cell. The antheridium represents a male organ, and its contents represent the essential substance of spermatozooids, or the fertilising matter of a pollen tube; and, after fecundation, the oospores answer to impregnated ova or fertilised seeds.

The oosporangium may burst and give exit to the oospores, or it may fall with them to the bottom. And, as a general rule, the oospores remain for a long time, sometimes several months, unchanged. Sooner or later, however, they germinate; and this process may take place in various ways:

1. The contents of the oospore may divide directly into locomotive zoospores which are set free.
2. The oospore may send out a hypha, the apical part of which becomes converted into a zoosporangium.
3. The oospore may send out a hypha, and this coming into contact with the body of a fly or some such matter, may develop into a mycelium in the ordinary way.

The whole series of phenomena now described represents the fullest set of changes known to occur in any one form of *Saprolegnia*. But, even in the same form, the series



may present notable variations. Thus, the zoospores may germinate without passing into an active condition ; or they may germinate immediately after they assume the first quiescent state. Again, in one and the same form, antheridia are sometimes developed and sometimes absent. In some forms, indeed, antheridia never make their appearance and consequently fertilisation does not occur. Nevertheless, the unfertilised oospores germinate and produce new *Saprolegniæ*, apparently just as well as if they were fertilised.

The commonest species of *Saprolegnia* has received the name of *S. ferax*, and both Pringsheim and De Bary agree that several so-called species, namely, *S. monoica*, *S. thureti*, and *S. torulosa* are merely more or less permanent varieties of *S. ferax* ; that they are all, in fact, members of the *S. ferax* group.

It has been seen that the fungus which grows on diseased salmon is unquestionably a species of the genus *Saprolegnia* ; and it is commonly identified with *S. ferax*. But this identification has rested upon very slender grounds. It is practically almost impossible to determine the species of a *Saprolegnia* until the characters of its oosporangia and of its antheridia (if it have any) have been accurately made out. At present not only are we without any sufficient account of these organs in the salmon *Saprolegnia*, but it is certain that they are, at most seasons, extremely rare. Mr. Stirling<sup>1</sup> speaks of having observed only four in the course of all his investigations ; and not a single specimen has presented itself in the considerable number of diseased salmon from the Conway, the Tweed, and the North Esk, which have come under our observation during the last four months.

When our inquiries commenced there was, strictly speaking, no proof that the salmon *Saprolegnia* could live on anything but a salmon. It was therefore quite possible that, since there are many species of *Saprolegnia*, that of the salmon might be peculiar to it, just as, in the analogous

<sup>1</sup> Mr. Stirling's valuable contributions to our knowledge of the salmon disease are contained in the 'Proceedings of the Royal Society of Edinburgh for 1878 and 1879. Mr. W. G. Smith, in a paper on the salmon disease in the 'Gardener's Chronicle,' May 4, 1878, not only affirms that "the resting spores are common enough," but figures them. However, Mr. Smith's figures of the zoosporangia are so unlike anything ordinarily observed in the salmon *Saprolegnia*, and his statement that "the fungus has invariably vanished with the death of the fish," is so strangely contrary to common experience, that it is difficult to know how much weight ought to be attached to his observations.



case of the potato disease, *Peronospora infestans* is different from all the species of *Peronospora* which abound upon European wild plants, and will not live on them, any more than these other species will live on the potato.

However this may be, it is easily proved that the *Saprolegnia* is not dependent on living salmon. In fact, if a patch of diseased skin is cut off and placed in a vessel of water, it will be found, in twenty-four hours, to be covered with a new growth of young hyphæ, close set and of nearly equal lengths, so that the surface resembles a miniature cornfield. A piece of the diseased membranous valve of the mouth of a salmon was placed in water on the 4th of March, 1882; on the 6th it was covered with young hyphæ one fifth of an inch long; and on the 7th these had elongated and developed multitudes of zoosporangia.

Moreover, there is not the least difficulty in proving that the salmon *Saprolegnia* is not dependant on salmon at all, but that it is capable of living on dead insects and pieces of wet bladder. If a recently killed fly is gently rubbed two or three times either over a fresh patch of diseased salmon skin, or over one which has developed the fresh growth just mentioned, and then placed in a vessel of water by itself, it will be found, in the course of eight-and-forty hours, to be more or less extensively beset with short, delicate, cottony-looking filaments, which rapidly increase in length and in number until, at last, the fly's body is enclosed within a spheroidal coat half an inch in diameter. These filaments are hyphæ having exactly the same size, form, and structure as those of the salmon *Saprolegnia*; their ends give rise to zoosporangia of the same character; and these produce zoospores of the same size, which germinate in the same way.

Between December, 1881, and April of the present year, repeated experiments of this kind have been made with diseased salmon from the Conway, the Tweed, and the North Esk, upon dead flies and small pieces of wet bladder, always with the same result. There appears, therefore, to be no doubt that the *Saprolegnia* of the salmon, like other *Saprolegniæ*, is capable of living and flourishing on a variety of dead animal matters.

When the *Saprolegnia* is established on one such substance it is easy to transmit it to another. The *Saprolegnia* obtained from diseased salmon was thus cultivated for many weeks (from the end of December, 1881, to the first week in April, 1882) in the hope of obtaining the oosporangia and thus identifying it with one or other of the described forms

of the *S. ferax* group. Up to the last-mentioned date, however, no oosporangia appeared on any of these cultures. The course of events was this: for two or three days zoosporangia were very abundant, and thousands of zoospores were set free. But, in no case which came under observation, for several months, were these zoospores provided with cilia, or actively locomotive. They were discharged from the zoosporangia as simple spherical corpuscles, which flowed passively away, and were very often seen germinating by sending out a single delicate hypha. Immense numbers of these spores accumulated among the hyphæ.<sup>1</sup>

After this condition had lasted for a day or two, the ordinary zoosporangia diminished in number, and "dictyosporangia" made their appearance in place of them. In other words, the spores, instead of being discharged, were retained within the zoosporangium, and began to germinate in that position.

At the same time the protoplasm accumulated in certain regions of the hyphæ, which often became swollen, and these accumulations were shut off from the rest by transverse partitions. The hyphæ thus assumed a jointed or beaded appearance, as in the *S. torulosa* of De Bary, and the joints might eventually separate from the intervening empty parts of the hyphæ as a sort of buds or *gemmæ*, which, after detachment, might begin to germinate by throwing out delicate hyphæ at one or many points. Sometimes these buds were terminal and spheroidal and closely simulated oosporangia, but they did not give rise to oospores. No trace of antheridial branchlets was ever visible.

In the third week of April, however, oosporangia and antheridia, in all respects similar to those of the "*monoica*" form of *Saprolegnia ferax*, made their appearance in a copious growth of the fungus on a fly, which was infected on the

<sup>1</sup> Among previous observers, Mr. Stirling and Mr. W. G. Smith describe and figure locomotive zoospores as if they were of ordinary occurrence. Mr. Brook, on the other hand ("Notes on the Salmon Disease in the Esk and Eden," 'Transactions of the Botanical Society of Edinburgh,' 1879), appears never to have seen locomotive zoospores; and Mr. George Murray, of the Botanical Department of the British Museum, who has been kind enough to make a series of observations and experiments, continued over six or seven weeks, on crops of *Saprolegnia*, raised upon dead flies infected from Conway salmon, has met with the same negative results. Quite recently, however (March 16), locomotive zoospores have been emitted from one of our specimens of salmon fungus cultivated on bladder. But, as in our specimens, so in those cultivated by Mr. Murray, no trace of oosporangia had appeared up to that time.

24th of March from a culture on bladder, which was again derived from a fly infected directly from a North Esk salmon on the 14th of March.

It may be safely concluded, therefore, that the salmon fungus is not a parasite peculiar to that fish, but that it is a form of the *Saprolegnia ferax*, which, so far as our observations go—and it must be remembered that these extend over only the quarter of the year between Christmas and the spring equinox—remains devoid of oosporangia so long as it infests the fish, and tends to persist in this condition for a long time, even when it is cultivated on those matters upon which the *Saprolegnia* more usually subsists. Future observation must determine whether oosporangia are developed on the *Saprolegnia*, while still growing on salmon, later in the year. The evidence of the fact at present extant is extremely unsatisfactory; and it is a remarkable circumstance that the figures which have been published show no trace of antheridial filaments.

That living fish may be attacked and destroyed by epidemic diseases, of which a *Saprolegnia* is either the cause or the constant accompaniment, has been known for a very long time.

Forty years ago the eminent German botanist, Unger,<sup>1</sup> described a disease which broke out among some carp in a pond in the Botanic Gardens at Gratz, and was obviously caused by a fungus, at that time known as *Achlya prolifera*, but which the description and figures given by Unger clearly prove to belong to the genus which is now distinguished as *Saprolegnia*, and, indeed, to be very similar to, if not identical with, *S. ferax*. More or less distinctly circumscribed pale spots appeared upon the skin of the back and of the fins. The fish became sluggish and sought the surface of the water. A velvety investment, formed of very delicate, colourless, close-set threads, showed itself on the spots affected, which rapidly became confluent, and extended from mouth to anus, and even on to the gills. The scales of the affected parts became detached, red, and swollen, and sometimes ulceration occurred. The animals could no longer move without appearing to suffer great pain; they remained at the surface of the water, lying either on their backs or on their sides, and death took place in eight-and-forty hours. Unger found that the disease could be transferred to perch by inoculation.

Again, there seems no reason to doubt that the fungus

<sup>1</sup> "Sur l'*Achlya prolifera*," 'Annales des Sciences Naturelles, Botanique,' serie iii, 1844.



which accompanied the epidemic disease affecting roach, dace, gudgeon, small pike, and perch, at Ightham in Kent, of which a very full and interesting account is given by Mr. Stirling,<sup>1</sup> is to be referred to *Saprolegnia ferax*. Here, however, ulcerative destruction of the skin does not appear to have occurred, and the mortality is said to have arisen from suffocation, the fungus obstructing the respiratory passages.

Pike kept in aquaria not unfrequently become covered with a fungus. The fish do not appear to be inconvenienced, and the fungus is very easily washed off. In a case of this kind, which we recently examined, the fungus was a *Saprolegnia*, the mycelium and the zoosporangia of which were altogether indistinguishable from those of the salmon fungus. Moreover, the hyphæ burrowed in the epidermis and distorted the cells with which they came in contact in just the fashion described below. As it was not desirable to kill the fish, it was impossible to determine whether the derma was penetrated or not; but the absence of sores and the ease with which large flakes of epidermis, in which the *Saprolegnia* was rooted, could be detached, lead to the conclusion that the *Saprolegnia* had not penetrated beyond the epidermis. The zoosporangia of the *Saprolegnia* taken from the fish emitted actively locomotive zoospores, but no oosporangia could be detected.

Dead flies infected with this *Saprolegnia* on the 18th of March, 1882, yielded an abundant growth, quite similar to that obtained in the same way from the salmon *Saprolegnia*; and on the 24th, that is, in six days, the characteristic oosporangia and antheridia of *Saprolegnia ferax* (*monica*) made their appearance.

It appears, therefore, that *Saprolegnia ferax* is capable of attacking a great variety of fishes during life, but that the concomitant pathological phenomena differ in different fishes.

Mr. Stirling's experiments on the transmissibility of the salmon fungus to other fish yielded only negative results. Diseased salmon skin was put into a vessel containing minnows, which nibbled the skin, and were none the worse. Experiments of this rough-and-ready sort, however, really prove nothing; and a great deal of light will assuredly be thrown upon the whole question of the salmon disease by carefully conducted experimental investigations.

At the present moment, we possess evidence that at least three distinct affections of the skin of fresh-water fishes have

<sup>1</sup> "Additional Observations on Fungus Disease of Salmon and other Fish," 'Proceedings of the Royal Society of Edinburgh,' x, 1879.



been confounded together under the name of "Aquarium fungus." One of these is associated with a *Saprolegnia* identical with that which attacks salmon; another is attended by the very closely allied fungus, *Achlya*; while the third is not accompanied by the growth of any fungus, but is a very curious morbid affection of the skin itself, apparently allied to epithelioma. We have hitherto observed it only in carp, the head, body, and fins of which sometimes appear covered with white patches, which present a most deceptive resemblance to those caused by *Saprolegnia*, the more especially as the edges of the fins may be eroded, and ragged fragments hang from the white patches. These patches, however, contain no fungus, but result from the abnormal growth of the epidermis, sometimes to eight or ten times its ordinary thickness, not unfrequently accompanied by a corresponding elongation of the papillæ of the derma.

Having thus dealt with the question of the nature and affinities of the fungus which is the constant concomitant of the "salmon disease," the next point for consideration is the relation of the fungus to the affection of the skin. Is the growth of the fungus the cause of that affection, or does the fungus merely find a favorable nidus in the production of the affection?

The *Saprolegniæ*, as we have seen, habitually grow on dead animal and vegetable substances; and it is therefore a fair supposition that some morbid affection may cause the local death of the skin of the fish; and that the fungus simply implants itself in the dead tissue, as if it were the dead body of a fly.

On the other hand, our knowledge of the destructive epidemics caused by *Empusa* in flies, *Botrytis* in silkworms, and *Entomophthora* in other caterpillars, and of the multifarious fungi which produce bunt, smut, and mildew in plants, affords at least equal ground for the supposition that the ulceration and destruction of the skin are caused by the invasion of healthy fish by the *Saprolegnia*. The decision of this question is obviously of the greatest importance.

Direct experimentation by infection of healthy salmon in the manner in which dead flies were infected from the diseased salmon being out of the question at present on account of its practical difficulties, the only profitable way of investigation lay in the study of the minute structure of the healthy and of the diseased skin, so as to ascertain the exact relation of the fungus to the morbid appearances.

The skin of the salmon, like that of vertebrated animals in general, consist of a superficial, cellular, non-vascular scarf-

skin, or *epidermis*, covering a deep fibrous and vascular true skin, or *derma*. The former is divisible into a superficial, a middle, and a deep layer of cells, the last being in immediate contact with the derma. The deep cells are vertically elongated, the middle ones more or less broadly spindle-shaped or rounded, while the thin superficial layer consists of flattened cells. The deep cells are constantly multiplying by fission, and their progeny become middle cells, the outermost of which, for the most part, becoming flattened, give rise to the superficial layer, which is continually shed and replaced. Some of the cells of the middle layer, however, enlarge, take on a more or less spheroidal form, and become filled with a mucous fluid. As they rise to the surface, they open and pour out this fluid, which lubricates the surface of the fish. In any vertical section of a properly prepared portion of salmon skin more or fewer of the openings of these cells are to be seen. The derma is composed of matted bundles of connective tissue, traversed by blood-vessels and nerves, and containing numerous lymphatic spaces. The superficial layer of the derma contains a number of dark pigment cells, of which there is a close-set zone immediately beneath the epidermis.

In a thin vertical section of the skin of the head of a salmon, which has passed from the sound skin through the centre of a diseased patch, the various structural elements which have been described, disposed with great regularity, are alone visible in the healthy part of the section. But, on advancing within the margin of the diseased area, hyphæ of the *Saprolegnia* are seen to penetrate horizontally between the cells of the middle layer, thrusting them asunder with so much force that the cells become bent and distorted, and adhere to a hypha as if they were spitted on it. And, in fact, it is because bundles of such hyphæ are thrusting themselves in this manner, as the roots of an ordinary plant thrust themselves into the soil, between the epidermic cells, that the radiating ridges which appear on the marginal area of the diseased patch are formed. Close up to the free ends of these hyphæ, however, the epidermis is perfectly healthy; and this fact suffices to prove that the growth of the fungus is the cause of the morbid affection of the epidermis, and not its consequence.

Proceeding further towards the centre of the diseased patch, the hyphæ become more numerous and take a vertical as well as a horizontal direction. Of the vertical ones, some traverse the epidermis outwards, thrusting aside and disturbing its cells, and terminating in short free ends on the

surface. Others of the vertical hyphæ, on the contrary, are directed inwards; and, as root-hyphæ, not only traverse the deep layer of the epidermis, but pierce the superficial layer of the derma, and penetrate into its substance for a short distance. Yet nearer the centre the epidermis is completely broken up into fragments and detached cells, which lie in the meshes of the thick mycelium formed by the horizontal and vertical stem-hyphæ of the fungus. The vertical stem-hyphæ attain their full length, often branching, and begin to develop zoosporangia. Towards the derma the root-hyphæ are so numerous and close set that they are often separated by interspaces which hardly exceed their own diameter where they penetrate the superficial layer of the derma. Moreover, they branch out in the latter to a depth of a tenth of an inch, often penetrating the bundles of connective tissue. Their ultimate ramifications usually end in curiously swollen extremities. Still more towards the centre of an ulcerated patch, the place of the epidermis is taken by the felted mycelium of the *Saprolegnia*, the superficial layer of the derma has disappeared, small vessels have often been laid open, and blood has been effused.

All these appearances become perfectly intelligible if we suppose that, when *Saprolegnia* spores reach the surface of the body of a live salmon, they behave in the same manner as we know they do when they reach the surface of the body of a dead fly. If it should light upon one of the apertures of the mucous cells an easy road into the soft interior of the epidermis is open to the hypha of the germinating spore. But, apart from this, the flat superficial cells are certainly as easy to pierce as is the tough cuticle of a fly. No doubt, as in the fly, the hypha grows directly inwards, and piercing the superficial layer of the derma, comes into direct relation with the abundant nutriment it finds there. The fungus then ramifies, on the one side, in the derma, on the other in the epidermis, sending off in the latter vertical branches which soon develop sporangia, and horizontal branches, which are driven, like subsoil ploughs, into the middle layer of cells. The zoosporangia emit multitudes of zoospores, many of which are deposited on the epidermis in the neighbourhood of the first; and, penetrating it in the same way, add to the *Saprolegnia* plantation. Thus the disease constantly spreads centrifugally; and, as the oldest and most luxuriant growth of *Saprolegnia* is in the centre, so is the mechanical destruction of the epidermis first effected there. But it is in this region also that the greatest number of root-hyphæ penetrate the derma. They cannot fail to interfere



with the nutrition of the tissues which they traverse; in fact, their ramifications are often so close set that the proper tissues of the superficial layer of the derma almost disappear. Sooner or later, therefore, necrosis sets in, and then ulcerative sloughing takes place, resulting in an open sore. No doubt the morbid process thus described is accelerated and intensified by the irritation caused by the innumerable small grains of sand and other foreign bodies entangled by the mycelium. But that the primary cause of all the mischief is the parasitic fungus does not appear to be open to doubt. If it were otherwise, the structural alteration of the skin should precede the fungus, and not follow it, as it actually does.

In fact, the *Saprolegnia* is the cause of the salmon disease exactly as the closely allied fungus, *Peronospora*, is the cause of the potato disease. In symptoms, progress, and results there is the closest analogy between the two maladies. *Peronospora*, like *Saprolegnia*, gives rise to spores which may be ciliated and actively locomotive, or may germinate without passing through an active stage. When these spores germinate on the surface of a healthy potato plant, their hyphæ perforate the walls of the cells with which they are in contact, and then ramify, as a mycelium, in the inner substance of the plant, carrying destruction wherever they go. The mycelium gives off hyphæ which pass through the stomates to the surface, and there they throw off abundant spores, which repeat the process until the whole plant is destroyed. Even the tubers are invaded; but in them the mycelium becomes quiescent on the approach of the winter season, to break out again, in full vigour, if the tubers are planted in the following spring. Moreover, there is as much uncertainty about the occurrence of antheridia and oosporangia, and of any sexual method of reproduction in the *Peronospora* of the potato, as in the *Saprolegnia* while it infests the salmon.

There is a great deal of reason to believe that the *Saprolegnia* growing on salmon is killed by salt water; and that the injured skin may heal and become covered with a new epidermis when a diseased salmon enters the sea. But the discovery that the root-hyphæ of the *Saprolegnia* ramify in the derma where the sea water cannot reach them, raises a curious and important question. It becomes possible that a diseased salmon returning to the sea may regain a healthy epidermis and appear perfectly sound; but that, like a potato-tuber invaded by *Peronospora* just before the approach of winter, the fungus in the derma may simply lie dormant,



and be ready to spring into activity as soon as the fish returns to fresh water. Cases of the appearance of the disease in quite fresh-run fish are occasionally reported, which would be readily explicable should this supposition turn out to be well founded.

Another possibility was suggested by the same fact. We know that the spores of the *Empusa*, a fungus which attacks living flies, germinate and bore through the cuticle in much the same fashion as the *Saprolegnia* enters dead flies. But the hypha of the *Empusa*, which has thus entered the fly, immediately breaks up into short joints, which diffuse themselves through the body of the fly and everywhere multiply by division, until they have appropriated all the nutritious matters which are available to them. It was therefore justifiable, on analogical grounds, to suppose that the hyphæ of *Saprolegnia*, which had entered the derma of a salmon, might break up in a similar way; and that the segments might be conveyed through the lymphatic and blood-vessels into all parts of the body, and either produce blood poisoning by a septic fermentative action, or develop centres of obstruction by lodgment in the narrower channels of the vascular system. However, there is no evidence to justify this suspicion. The hyphæ in the derma show no signs of division, nor have any toruloid bodies or other structures that can be regarded as derivatives of *Saprolegnia* been observed, either in the blood or in any of the viscera.

The salmon disease, in fact, appears to be a purely cutaneous affection; and the fish seem to die partly from irritation and consequent exhaustion, and partly, perhaps, from the drain on their resources, caused by the production of so large a mass of vegetable matter at their expense.

The opportunities for the investigations, the chief results of which have now been detailed, have arisen only during the last three or four months; and a great deal more time and attention must be devoted to the subject before it can be expected that many of the obscurities and difficulties which still hang about it can be cleared up.

It is needful to discover the conditions under which the fungus exists in those rivers which are infested by the disease when the full-grown salmon have deserted them; whether it lingers in isolated cases among the parr, trout, or the non-salmonoid fish; or whether it contents itself with the bodies of dead insects and other dead animal, and perhaps vegetable, substances; or whether, in the late summer, oosporangia may not be formed and give rise to oospores, which, as De Bary's experiments show, may have a dormant

period of three or four months, that is to say, sufficient to preserve them till the next return of the salmon.

On all these points persons conversant with the use of the microscope, who are resident in the neighbourhood of salmon streams, might obtain information of great value, hardly to be procured in any other way.

Although all the evidence leads to the conclusion that the *Saprolegnia* is the immediate and primary cause of the salmon disease, and that, in the absence of the fungus, the disease never makes its appearance, however polluted the water may be, or however closely the fish may be crowded; yet, in this, as in other epidemics caused by parasitic organisms, the prevalence and the mortality of the malady, at any given time and in any given place, must be determined by a multitude of secondary conditions independent of the immediate cause of the disease.

In the case of the potato disease it is well known that dry weather is extremely unfavorable to the growth and diffusion of the *Peronospora*. In such a season a plant may be affected here or there, but cases of disease are so rare that they escape notice. But, if even a few days of rain with a thoroughly damp atmosphere supervene, the fungus spreads from plant to plant with extraordinary rapidity, and field after field is devastated as if struck by a sudden blight. So with the epidemic disorders of mankind. In a large town isolated cases of smallpox, measles, diphtheria, and the like, constantly occur, and every case is the source of a vast quantity of infectious material. Nevertheless, it is only under certain conditions that this infectious material takes effect and gives rise to an epidemic.

At a moderate estimate the *Saprolegniæ* on a single dead fly may carry a thousand zoosporangia. If each sporangium contains twenty zoospores, and runs through the whole course of its development in twelve hours, the result will be the production of 40,000 zoospores in the course of a day, which is a number more than sufficient to furnish one zoospore to the cubic inch of twenty cubic feet of water. Even if we halve this rate of production it is easy to see that the *Saprolegniæ* on a single fly may yield a sufficient abundance of zoospores to render any small and shallow stream, such as salmon often ascend for spawning purposes, dangerous for several days. For a single one of these spores, if it adheres to the surface of the skin of a salmon and germinates, is sufficient to establish the disease. Other things being alike, of course the greater the quantity of *Saprolegnia* in a stream the greater the chances of infection for the fish which enter it.

In looking for the causes of an epidemic of salmon disease we have, therefore, to inquire, in the first place, into the conditions which favour the growth of the *Saprolegnia*. It is known that the *Saprolegniæ* subsist, not only on dead insects and on dead crustacea and molluscs, but on some other dead animal matters and on decaying plants. The particular form which infests the salmon, as we have seen, flourishes as well upon dead flies; it can also be grown upon pieces of bladder, but whether it can be transferred to decaying vegetable substances has yet to be determined.

Hence it follows that, within certain limits (active putrefaction appearing to be unfavorable to *Saprolegnia*), an increase of the quantity of dead insects and other such organic matters in a river must tend to favour the growth and multiplication of any *Saprolegniæ* which it contains, and hence to increase the liability to infection of the salmon which ascend it.

And that this is no mere hypothetical deduction is very well shown by a remarkable case which was carefully investigated by Goeppert<sup>1</sup> nearly thirty years ago.

A peculiar water-mould, commonly known as *Leptomitius lacteus*, but which is so closely allied to *Saprolegnia* that Pringsheim places it in that genus, is widely spread in running waters, where it grows on all sorts of dead organic substances.

A factory for making spirit from turnips was established near Schweidnitz, in Silesia, and the refuse was poured into an affluent of the River Westritz, which runs by Schweidnitz. The result was such a prodigious growth of *Leptomitius* that the fungus covered some 10,000 square feet at the bottom of the stream with a thick white layer, compared to sheep's fleeces, choked up the pipes, and rendered the water of the town undrinkable. Scattered hyphæ of this *Leptomitius* may sometimes be found among those of *Saprolegnia*, growing on fresh-water fishes; and the two forms are altogether so similar, that conditions analogous to these which stimulate the growth of the one may safely be assumed to favour that of the other.

Brefeld has pointed out that there is no better medium for the culture of fungi of all sorts than an infusion of dung ("mistdecoct"). Land under high cultivation undoubtedly supplies the waters in its neighbourhood with something that nearly answers to an infusion of dung; and this must be taken into account in discussing the possible factors of salmon disease.

<sup>1</sup> 'Botanische Zeitung,' xi, p. 163, 1853.



Again, it is known with respect to many of the common moulds, such as *Penicillium* and *Mucor*, which are habitually *saprophytes* (that is to say, live on decaying organic matter, as *Saprolegnia* does), that they flourish in certain artificial solutions containing salts of ammonia. It is quite possible, though whether the fact is so will have to be experimentally determined, that *Saprolegnia* is capable of living under the same conditions. Fungi are also extremely sensitive to slight differences in the acidity or alkalinity of water, so that even apparently insignificant changes in this respect may come into play as secondary conditions of salmon disease. Hence, although there is not the slightest ground for regarding "pollutions," whether they arise from agricultural or from manufacturing industries, as primary causes of salmon disease, they may have a most important secondary influence; they may, in fact, determine whether, in any river, the disease shall be sporadic or epidemic.

But of all the conditions which determine the increase of *Saprolegnia*, and therefore multiply the chances of infection of healthy fish, the presence of already diseased fish is obviously one of the most important. A large fully diseased salmon may have as much as two square feet of its skin thickly covered with *Saprolegnia*, and its crop of spores may be taken as equivalent to that of several hundred flies. It may be safely assumed that forty such salmon might furnish one spore to the gallon for all the water of the Thames which flows over Teddington Weir (380,000,000 gallons) in the course of a day.

In 1878, 350 dead salmon were taken out of a very small river, the Esk,<sup>1</sup> in three days. If the zoospores which these gave off had been evenly diffused through the water of the Esk, the difficulty is to understand how any fish entering it could escape infection.

In fact the objection easily arises that these arguments prove too much; and that, if the *Saprolegnia* is the cause of the disease and its spores thus widely diffused in an infected river, not a fish which ascends that river should escape the disease.

But such an objection loses its force if it is remembered that, though the *Saprolegnia* is the cause of the disease and though a single spore is undoubtedly sufficient to kill a salmon, yet, in order to produce that effect, the spore must, in the first place, reach and adhere to the epidermis of the salmon; in the second place, it must germinate; and, in the

<sup>1</sup> Stirling, 'Proceedings of Royal Society of Edinburgh.' vol. ix, p. 726.



third place, the delicate hypha which it sends out must bore its way through the epidermis into the derma.

Each of these conditions of successful infection may be modified in endless ways of which we know nothing—by the state of the epidermis of the fish; by the motility and the general vital energy of the spore; by the composition of the water, and especially by that of its gaseous and acid or alkaline contents.

To take only one of these conditions. If the spores germinate within the zoosporangia, or are not locomotive after they leave it, their chances of diffusion, and hence of receiving a healthy fish, will be vastly less than if they are locomotive, for even a short time. And, again, their chances will be far less if they germinate after the first locomotive state, which lasts only a few minutes, than if they enter into the second locomotive state, which may endure for four-and-twenty hours or more. So, if the salmon *Saprolegnia* produces oosporangia in the late summer, and these lie dormant at the bottom until the following spring, the chances of infection of fresh-run fish will be greater than they will be if the continuance of the existence of the *Saprolegnia* through the winter depends upon the accident of a sufficient supply of dead organic substances.

Moreover, any one who has practised the cultivation of *Saprolegnia* is familiar with the difficulties which arise from the swarms of *Infusoria* and *Bacteria* which devour, or otherwise destroy, the fungus, notwithstanding all his efforts to preserve it.

The struggle for existence rages among fungi as elsewhere; and the question whether a salmon which enters water in which *Saprolegnia* is present shall be infected or not depends upon the mutual adjustment of a vast variety of conflicting agencies. Until we have learned something more than we at present know of these agencies, and of the history of the salmon *Saprolegnia* itself, there can be no thoroughly safe foundation for any view which may be put forward as to the best mode of dealing with the disease.

Nevertheless, since it is evident that every diseased salmon which remains in a river must immensely increase the chances of the infection of the healthy fish in that river, the policy of extirpating every diseased fish appears, theoretically, to be fully justified. But whether, in endeavouring to carry such a policy into effect in any given river, the cost would not exceed the loss from the disease, is a point which must be left for the consideration of Boards of Conservators:

## NOTES AND MEMORANDA.

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**On Certain Methods of Cutting and Mounting Microscopical Sections.**—It is many years since Leuckart first suggested the cutting of thin slices of an animal and mounting them as transparent objects. Now it is possible to cut a worm into sections from end to end and be sure that every section is perfect, or to obtain a series of fifty sections from the morula of a Medusa but one hundredth of an inch in diameter.

The methods I am about to describe are modifications of older processes and constitute the latest stage of development of the section-cutter's art.

*Hardening.*—Any of the ordinary hardening methods may be used, but it is essential that all trace of acid should be removed in order to obtain good staining results. Corrosive sublimate is an exceedingly useful hardening reagent, and tissue treated with it stains as readily as if treated with alcohol only. The solution used is a concentrated one, the fresh tissue or living animal is placed in it, for 15 to 30 minutes according to its size, it is then washed in water and transferred to alcohol of 50 per cent.: a large relative bulk of this must be used and the tissue well permeated by it, otherwise some corrosive sublimate is left in the tissue and is thrown down in needles when strong alcohol is added.<sup>1</sup> After 24 hours the tissue is transferred to alcohol of 70 per cent., and after 24 hours to alcohol of 90 per cent. and then to absolute alcohol. With large pieces of dense tissue this should be changed once or twice. After two or three days the tissue is ready for staining. If time is an object and no acid has been used in the hardening, the tissue may be transferred directly from alcohol of 70 per

<sup>1</sup> We owe this, and many other excellent histological methods, to Dr. Arnold Lang, of the Zoological Station at Naples.

cent. to the staining fluid, but it is advisable and in the case of delicate tissues necessary to complete the hardening process before staining.

*Staining.*—Grenacher's alcoholic borax carmine is used. Pure carmine, 2.5 per cent., is added to a solution of borax 4 per cent (in water), this is allowed to stand for two or three days and occasionally stirred; the greater part of the carmine will dissolve. To this solution is added an equal bulk of alcohol of 70 per cent. This mixture must stand for a week and then be filtered, when it is ready for use. If on keeping more carmine is deposited it should be refiltered.

The tissue is placed in this solution and allowed to remain one, two, or three days according to its size; it is hardly possible to overstain, and there is sufficient alcohol in the solution to prevent injury to any but the most delicate tissues. For such tissues a solution can be prepared containing more alcohol but of course less carmine.

The tissue when removed from the staining fluid is placed in alcohol of 70 per cent., acidulated with hydrochloric acid (3 to 6 drops of the acid to 100 c.c. of spirit). This dissolves out all excess of carmine and fixes the rest. The tissue a dark purplish red when taken out of the borax carmine, should be left in the acidulated alcohol till it acquires a bright transparent look (3 to 6 hours), it may then be transferred to absolute alcohol and afterwards to turpentine. When thoroughly permeated with this latter (the time necessary varying as the size of the lump of tissue) it is ready for imbedding.

*Imbedding.*—This is done in paraffin and it is exceedingly important to obtain a suitable paraffin. It should melt at 100° or 115° F. Paraffins of various melting points should be kept in the laboratory; they may be purchased at the dealers.

The tendency to curl up on the part of the section may be reduced to a minimum by obtaining a paraffin of the proper consistency, but this seems to vary according to the temperature of the room in which the sections are cut.

The paraffin must be melted in a small covered vessel in a water oven, great care being taken to keep it in a dry atmosphere. The temperature in the oven should never rise more than two or three degrees above the melting point of the paraffin used.

When the paraffin is melted the tissue is removed from the turpentine and placed in it, and this must be kept at its melting point for some hours until the tissue is thoroughly

permeated, it may then be poured into a paper trough, watch glass, or other vessel and allowed to cool.

*Cutting.*—There are two forms of microtome suitable for cutting sections in series. In one the tissue is raised directly by a fine screw and the sections cut with an ordinary razor: in the other form, now so largely used, the tissue in its holder is moved up an inclined plane and the sections cut with a large knife which works backwards and forwards in a horizontal slot running parallel to the inclined plane. In both cases the machine is fixed to the table or heavy enough to remain steady, so that while the razor is worked with one hand the other hand is at liberty to hold a little paper spatula—a small piece of paper run on at the end of a small scalpel—to prevent the sections curling. The paraffin block is pared down to the smallest size possible, and, as the razor is drawn along, the edge, which commences to curl, is caught by the paper and prevented from so doing; the section is then transferred to the slide.

*Preparation of the slide.*—The slide is smeared with a strong solution of shellac in anhydrous creosote. Care must be taken to have as little as possible on the slide. By this method the sections are stuck to the slide, thereby saving the most delicate objects from falling to pieces after the paraffin is removed and enabling one to mount numerous section on one slide. The importance and value of this treatment cannot be over-estimated. It enables one to mount with absolute certainty whole sections of the most friable objects, such as an insect, without a single fragment of the section becoming displaced.

*Mounting.*—The slide bearing the sections is now placed in a water-oven or on a tin box containing water at a temperature two or three degrees above the melting point of the paraffin used. The slide is left here for at least half an hour. The object of this warming is twofold, to evaporate the creosote and to melt the paraffin.

The slide is now taken up, and while the paraffin is still molten is flooded with turpentine dropped from a small pipette. This dissolves melted paraffin instantaneously, and precipitates the shellac fastening the sections to the slide. The turpentine is allowed to flow off and replaced by new until all the paraffin is removed. The slide is then allowed to drain, the edges wiped, and the cover glass put on. The Canada balsam, which should be very fluid, is placed on the under surface of the cover glass; this is turned over and quickly lowered. The balsam dissolves the shellac, and if the



cover glass is not put on very quickly the sections may shift or delicate sections come to pieces and float over the slide. It being necessary to use the balsam in such a fluid condition and a certain amount of turpentine always remaining upon the slide, the slides should be looked over the next day and more balsam added at the edge of the cover glass if necessary.

These methods, especially that of fastening the sections to the slide with shellac, although suggested and elaborated by zoologists for the purpose of mounting serial preparations, will, no doubt, come into very general use in ordinary histology for such tissues as placenta or spleen where very thin sections have always been found liable to fall to pieces and the most important pieces to fall out and be lost.

I have especially to thank the members of the staff of the zoological station at Naples for my knowledge of the above process which may, indeed, in its entirety be said to have emanated thence.

The hardening and staining processes I have described are those I have found to answer best for a large variety of objects, but they can, of course, be modified to suit special cases.—ALFRED GIBB BOURNE, B.Sc. (Lond.), University Scholar in Zoology and Assistant in the Zoological Laboratory, University College, London.

**The Central Duct of the Leech's Nephridium.**—Since describing the structure of the nephridia in the medicinal leech<sup>1</sup> I have devoted a considerable amount of time to the investigation of the comparative histology of the other Hirudinean genera which I have been able to obtain, and have made further observations on *Hirudo* which have led me to alter my conclusions as to one or two details.

Both picrocarmine and hæmatoxylin failed to thoroughly stain the nephridial cells, but with borax carmine I have succeeded in staining the peripheral portion of the cell and the nucleus.

The use of borax carmine has demonstrated the existence of *nuclei in the walls of what I have called the central duct*, showing the walls of this duct to be cellular and not merely cuticular as I had supposed.

I am indebted to Dr. Arnold Lang, of the zoological station at Naples, for pointing out to me that in *Aulostomum* the walls of this duct are formed of cells which, although in rather a degenerate condition, are easily seen.

<sup>1</sup> This Journal, vol. xx, July, 1880.

The "central duct" is continuous with the "vesical duct." The walls of the latter region are cellular, as I stated before. I have now found that the cells lining this vesicle duct are, like the other nephridial cells, perforated by ductules communicating with the ductules in the other regions of the gland.—A. G. BOURNE.

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## FRANCIS MAITLAND BALFOUR.

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WE have to record the death of our beloved friend and colleague, FRANCIS MAITLAND BALFOUR, Fellow of Trinity College, Cambridge, for many years a contributor to, and co-editor of, this Journal.

His death was caused by a fall whilst he was attempting the ascent of the Aiguille de la Belle Etoile, on the south side of Mont Blanc, on July 19th last.

He was only thirty-one years of age.

The blow to our science and to the University of Cambridge, which had just founded a Professorship to do him honour, is overwhelming, and can be estimated by those who have followed his work in these pages.

Great as is the public loss, there is a deeper grief for those who knew him as a companion and fellow-worker, honouring him for his great mental powers and the firmness of will with which he applied them, loving him for his chivalrous courtesy and for the faithful and earnest nature which shewed itself in all he said and did. These must sorrow not now only, but through future years, as day after day they feel the need of his aid and fellowship.

*September, 1882.*





## MEMOIRS.

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*On the DEVELOPMENT of the EUROPEAN OYSTER (*Ostrea edulis*, L.)* By Dr. R. HORST, of the Utrecht Museum of Zoology. (With Plate XXVII.)

LAST summer, when the Dutch Zoological Station was erected in the neighbourhood of the East Scheldt oyster-banks, I studied for some weeks the development of the oyster. Although this research is not fully completed, I believe that the following communication may contribute to augment our still very scanty knowledge of the embryology of Lamellibranchiata.

In studying the development of the oyster we meet with peculiar difficulties, which made the French zoologist—Lacaze-Duthiers—say justly, “L’hûître est certainement l’une des espèces du groupe des Acéphales lamellibranches la plus difficile à étudier dans son organisation comme dans son développement.”<sup>1</sup> The embryos not only pass their first stages of development within the pallial cavity of the mother, but the impregnation of the eggs takes place also internally instead of externally; perhaps the eggs and the spermatozoa even meet in the efferent ducts of the genital gland. To observe the first changes of the impregnated egg one cannot make use of artificial fertilisation, but one is obliged to open by chance several breeding oysters. Having opened a mother-oyster in the usual way, that is, by means of cutting the adductor muscle, it soon dies, and so the normal development of the spat which it contained is accordingly disturbed. One can keep the embryos alive for some days outside the mother in an aquarium, but not without their showing very soon either pathological states or the development

<sup>1</sup> “Mémoire sur le Développement des Acéphales lamellibranches,” ‘Compt. Rend. Acad. Sc.,’ Paris, t. xxxix, p. 1197.

simply ceasing. So Lacaze-Duthiers tells us that the oyster larvæ remained alive in his aquaria for longer than a month, but all the time he saw no important changes take place in their organisation, which is decidedly not normal. Though I succeeded a few times in following the development of the spat for some days undisturbedly by making a little hole in the margin of the shell, by means of which the oyster was not or hardly at all hurt, and in which way I could nevertheless reach the eggs with a glass tube; yet even this was only for a short time, because the oyster cast out at every artificial delivery a great quantity of embryos, and consequently soon lost all the spat. It is, therefore, not possible to obtain an unbroken series of the different stages of the larvæ, but we must take refuge in comparing the observed stages together, and try to form in that way an idea of the development.

Another difficulty is that the breeding oysters cannot always be recognised externally; the relaxation of the adductor muscle, and accordingly the less energetic closing of the shell, is to be sure a pretty certain sign that the oyster contains embryos, but this phenomenon happens most obviously, the older the spat and the closer to the period of its getting free. This may be the reason that I generally found more oysters with old spat than with young, and, therefore, the first stages of segmentation of the egg mostly remained unknown to me.

Davaine<sup>1</sup> has figured some of the earliest stages of segmentation of the egg of *Ostrea edulis*. These, combined with older stages observed by me (figs. 1 and 2), and with Brooks'<sup>2</sup> observations on the development of *Ostrea virginiana*, leave no doubt in my mind that the segmentation of the oyster egg in the beginning is quite similar to that of the egg of other Lamellibranchiata. The first stages I observed were provided with a large granular sphere at the lower (vegetative) pole of the egg, while at the opposite (formative) pole lay some smaller, clearer cells; a segmentation cavity seems not to exist (fig. 1). The smaller (epiblastic) cells are increasing rapidly in number and embrace the great hypoblastic sphere, though without closing it wholly in. The hypoblastic sphere begins to divide itself first into two great round cells (fig. 2), afterwards into several cylindrical cells (fig. 4); meanwhile the embryo has lost its spherical form, and has

<sup>1</sup> 'Recherches sur la Génération des Hûîtres,' pl. ii.

<sup>2</sup> "The Development of the American Oyster (*Ostrea virginiana*, List.)," 'Studies from the Biological Laboratory of John Hopkins University,' No. iv, 1880, pl. 1, 2 and 3.

assumed, in consequence of an invagination at the lower side, a reniform shape (fig. 3, the lower part is turned upwards)

By examining somewhat older embryos by section (fig. 4), it becomes clear that the layer of hypoblastic cells is a little invaginated, and so a gastrula has arisen. Since there is no segmentation cavity one cannot speak of a true invagination, and it is difficult to say if we have to do with an embolic or an epibolic gastrula. The last form seems to be characteristic for the other marine Lamellibranchiata. Indeed Rabl<sup>1</sup> and others have already shown that these two apparently fundamental different ways of gastrula formation are connected by a series of transitions.

The oyster embryo shows in this stage the remarkable peculiarity that not only the vegetative pole has an invagination, but that besides there is to be found a very evident pit at the opposite pole, a little way from the top. Examining the embryo from the side this invagination is quite striking (fig. 5, sk), and an optical section (fig. 4) teaches us that it has arisen by epiblastic cells, which have somewhat invaginated themselves inwards. By further development there arises here a pouch formed by high cylindrical cells, with a narrow lumen, whose blind end is turned to the dorsal pole of the embryo, whereas its opening is directed transversely to the longitudinal axis of the embryo (figs. 7 and 8). Surely this pouch is nothing but the *shell-gland*, as is shown by the examination of older stages. The assertion of Fol<sup>2</sup> that in *Ostrea* the shell-gland is not a true invagination, but rather a feeble excavation of the thickened epiblast, is therefore not quite exact, and probably founded upon the examination of older larvæ (observed by Salensky), in which the invagination afterwards everts and flattens out gradually, like that of other Mollusca embryos. The shell-gland, as we know, was first discovered<sup>3</sup> in the *Mollusca cephalophora*, and was afterwards observed also by Ray

<sup>1</sup> "Entwicklung der Tellerschnecke," 'Morphol. Jahrbuch,' Bd. v, p. 601.

<sup>2</sup> "Etudes sur le Développement des Mollusques," 'Archiv. de Zool. Experim,' t. iv, p. 180.

<sup>3</sup> [Dr. Horst is mistaken in stating that the shell-gland or præconchylian invagination was first observed in *Mollusca cephalophora*. It was discovered by Lankester in *Pisidium* in 1871, being previously altogether unknown, and its occurrence in the embryo of that Lamellibranch and of *Aplysia*, announced by him in the 'Annals of Nat. Hist.,' February, 1873, seven years before Dr. Hatschek's publication, and two years before that of Fol. In 1874 Lankester published in this Journal and in 1875 in the 'Phil. Trans.,' figures of the shell-gland in *Lymnæus*, *Neritina*, and *Aplysia*, as well as in the Lamellibranch *Pisidium*.—ED. 'Q. J. MIC. SCI.']



Lankester<sup>1</sup> and Hatschek<sup>2</sup> in some Lamellibranchiata (*Pisidium*, *Cyclas*, and *Teredo*). In comparison with these genera the shell-gland of the oyster appears at a very early period.

The first investigators of the development of the oyster—Davaine and Lacaze-Duthier—have mentioned already “*une échancrure*” and “*une depression*,” which gives the embryo a cordiform shape; the invagination of the shell-gland seems, therefore, not to have been unknown to them, but as they studied the embryo only on the surface they have not understood the true signification of that pit. According to Brooks’ observations, the embryo of *Ostrea virginiana* is provided also with a deep semilunar groove, which he nevertheless believes to be the blastopore. Comparing, however, his fig. 32 (op. cit.) with mine (figs. 5, 6, and 8), I think it very probable that what Brooks supposed to be the blastopore is nothing but the opening of the shell-gland. This interpretation quite agrees with his observation that on the point, where is situated his blastopore, he sees afterwards develop the first traces of the shell. A similar origin of the shell of Lamellibranchiata has till now only been found by Rabl<sup>3</sup> in the *Unio*, and is so contrary to the observations on the development of other Mollusca that it certainly deserves to be examined again. In a following stage, figured in fig. 6, we see the invagination of the hypoblast completed, forming the archenteron. Behind the blastopore lie a couple of large cells, probably the first mesoblastic cells, whose origin unhappily remained unknown to me. Meanwhile the ventral side of the embryo begins to project a little, forming a kind of foot, which makes the embryo strongly resemble a young Gasteropod. The blastopore is still clearly visible, and shows a somewhat triangular form. As far as I could see it does not disappear during the further development, and directly passes into the permanent mouth, or rather into the cardia. For just as in those embryos whose blastopore remains not open, and whose mouth and œsophagus are formed by an invagination of the epiblast, here also the epiblastic cells participate in the formation of the oral region of the alimentary tract.

During the further growth of the embryo great internal as well as external changes take place. The shell-gland

<sup>1</sup> “On the Developmental History of Mollusca,” *Phil. Trans. of the Royal Society*, 1875.

<sup>2</sup> “Ueber Entwicklungsgeschichte von *Teredo*,” *Arbeiten aus dem Zool. Institut*, Wien, t. iii, 1880.

<sup>3</sup> “Ueber die Entwicklungsgeschichte der Malermuschel,” *Jenaische Zeitschrift*, xi, 1878.



begins gradually to lose its original character of a glandular invagination, flattens out, and passes into a saddle-shaped thickening of the epiblast, consisting of long columnar cells (fig. 9 *sk*). The secretion of these cells gives origin to a thin cuticular membrane, which forms the rudiment of the shell, because in the full-grown animal the shell-hinge is situated on the same point; the assertion of Davaine—"Un trait transparent . . . . c'est le premier indice de la charnière"—was quite right. The bivalve shell of *Ostrea* is, therefore, undoubtedly an unpaired formation, and is not developed, as suggested by Lacaze-Duthiers, of two separated halves, which afterwards unite and form a hinge. According to Brooks' observations the shell of the American oyster also is formed at first of two separated valves, which develop from a small, irregular, transparent body, situated on both sides of the dorsal pit (his blastopore).

Considering, however, as I have demonstrated before, that the true signification of that pit and the real blastopore have probably escaped him, we may doubt a little the exactness of his investigations. Moreover, my observations so entirely agree with Hatschek's on the first apparition of the shell in *Teredo* that it seems permitted to conclude *that in all the Molluscs the shell is formed in the same way*.

With regard to the internal changes of the embryo we observe in fig. 9 that between the two layers of hypoblast and epiblast a space is formed, the body cavity, which contains several mesoblastic cells. In this stage also the velum makes its appearance, as a ring of cilia, situated in the præ-oral region, which consists of large cylindrical cells.

Afterwards the shell is increased rapidly in size, and covers a great part of the body of the larva (fig. 10); treated with sulphuric acid it becomes clear that it contains already calcareous salts. The velum develops more and more (fig. 11), and in the midst of the cephalic area, which it surrounds, a thickening of the epiblast appears (Scheitelplatte). The infundibuliform œsophagus leads to a pear-shaped stomach, while the intestine gets an external opening, leading into the pallial cavity.

At a later period (fig. 12) several parts of the larval body (the cephalic thickening, the œsophagus, the stomach) become provided with a reddish and black pigment, which gives the at first colourless larvæ (white spat) a greyish-blue colour (black spat). Beside the alimentary tract in the body-cavity there appear ramified muscular fibres, a ventral and a dorsal one (*vs* and *ds*), which originate beside the hinge and attach themselves at the inner side of the velum, which

they can retract into the shell. At the dorsal side another muscle is situated, passing transversely from the right valve to the left, and serves to shut the shell (*sp*). The præ-oral ciliated ring is composed of a double row of cilia, which surround a vaulted area, in the middle of which is found the thickening already mentioned before, now consisting of several layers of epiblastic cells (*tp*); this thickening gives rise to the supra-œsophageal ganglion. On its upper side it is traversed by a groove, which seems to divide it slightly into two halves.

Meanwhile the alimentary tract has augmented considerably in length and in width, the stomach is differentiated into a superior and an inferior division, and the intestine begins at the junction of these two divisions. The superior portion of the stomach gives rise at both sides to a large lobe (*e*), the origin of the liver. The digestive tube is covered with cilia on the whole of its inner surface, without, perhaps, including the hepatic sacs.

At the ventral side of the larva, almost in the region of the foot, a bur-shaped thickening of the epiblast is present; this is probably the rudiment of the pedal ganglia, although I have not found the otocysts, which Lacaze-Duthiers says he has observed; also I have not succeeded in discovering an excretory organ, though I did my utmost to find it, because the oyster-larva offers so much resemblance with the Trochophora of *Teredo*, described by Hatschek. Perhaps later investigations will show that provisional renal organs are not wanting here.

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RESEARCHES on the MORPHOLOGY and LIFE-HISTORY of a  
TROPICAL PYRENOAMYCETOUS FUNGUS. By H. MARSHALL  
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versity, late Cryptogamist to the Government in Ceylon.  
(With Plates XXVIII and XXIX.)

THE subject of the following memoir was discovered, during the wet season of 1880, in the Royal Botanic Gardens, Peradeniya, Ceylon, as an epiphyte, and, as will be shown, parasitic to a certain extent on the leaves of *Jasminium pubescens*, shrubs of which are cultivated in the above-mentioned gardens. At various times since I have observed it on other specimens of the same plant, but so far have no knowledge of its existence elsewhere.

During the early stages of its development on the leaves, the host-plant does not appear to offer any well-marked symptoms of disease; but, as the black specks of the fungus spread over the leaves and other parts of the plant, sufficient evidence of a "diseased state" is presented to warrant our regarding the presence of the parasite as the forerunner of a definite plant-disease, of which it must also be considered the sufficient cause.

The healthy and normal, broadly ovate or cordate leaf of the jasmine referred to, is of a soft, dull green colour, not reproduced in the smaller of the two figures at fig. 1,<sup>1</sup> which represents a young leaf on which the fungus is commencing to obtain a footing. The fungus itself is seen as delicate, black, cloudy, or dotted patches, chiefly on the upper surface of the leaf, but occurring also on the lower epidermis. As these clouds and blotches become extended, or after a period of persistence on a given area, the leaf turns paler, patches of yellow, or yellowish white, make their appearance, in-

<sup>1</sup> This excellent figure was drawn by Mr. W. De Alwis of Peradeniya, and I take this opportunity of thanking him for the trouble he has taken in its execution. All the other figures were drawn by myself. The natural colours have not been reproduced in the plates.

crease and spread, and the leaf becomes sickly or "diseased," and soon ceases to be of use to the plant.

If the black, cloudy mottling just described be examined with a good hand lens, or with a low power of the compound microscope, it is seen to consist of aggregations of dense black maculæ, united by networks of fine and beautifully branched dark lines. In some cases the whole surface of the leaf is thus covered; in others, the cloudy patches and networks are confined to particular areas. Closer examination shows that the denser and blacker spots consist of certain orbicular, boss-like bodies, from which the delicate branched network spreads in a more or less stellate manner, running into similar radiations from neighbouring bosses, by branchings and anastomoses of all degrees of complexity.

If the epidermis of the leaf, together with the reticulations, &c., described above, be carefully removed with a sharp razor, and examined by transmitted light, their several characters are determined still more easily; the deep, shining black colour, however, being softened to a rich vandyke-brown, tinged with olive. (*Cf.* fig. 2.)

Still closer investigation, with a higher power, shows that the dark bosses (which are of various sizes, from that of a small dot to the diameter of a small pin head) are closed and appear nearly homogeneous when young, but become open above by irregular radiating slits as they increase towards maturity, and disclose a cavity within the boss, from which spring masses of slender filaments and very delicate *asci*, containing *ascospores*, the whole embedded in a sort of gelatinous matrix (figs. 3 and 4). The networks of radiating, branching, and anastomosing filaments, also, are now seen to be composed of tubular and septate fungus-hyphæ, the thick walls of the long cells being strongly carbonised, rendering them—as also those composing the ascus-containing bosses—brittle and dark coloured. Here and there, united by slender stalks to the deep-coloured hyphæ of the network, may be seen peculiar hyaline and colourless *Haustoria*, dipping into the epidermal cells of the subjacent leaf (figs. 6 and 8), and anchoring the whole firmly thereon.

For the sake of simplicity and clearness, I propose to alter somewhat the order in which the following facts became discovered, and to describe the anatomy and development of this fungus systematically, from the germination of the ascospore on the young leaf in the rainy season.

The spore itself is a short, cylindrical, deep brown body, equally rounded at both ends, and slightly constricted in the



middle (fig. 5). In outline, therefore, it is oval or oblong. It presents a somewhat thick, strongly carbonised, brittle *exospore*, which gives it the dark colour, and which is externally studded with numerous, closely set, minute, stiff papillæ, which project perpendicularly from all parts of the surface; closely lining the inside of this is a thin *endospore*, scarcely recognisable through the dark, opaque outer wall. At that part of the spore corresponding to the slight indentation of the outline, a distinct, firm septum divides the whole into two equal halves. The granular protoplasm usually contains one or two nucleus-like oily drops, though no true *nucleus* could be discovered.

On sowing such a spore in water on the upper surface of the jasmine leaf, a blunt outgrowth from the end of each of the two chambers is soon established as a pale, granular-looking, blunt hypha, with a diameter equal to about one third that of the spore. This germinal hypha branches and forms one or two septa very soon after its emergence, and its walls then acquire the brown colour of the adult fungus. This hypha presents the ordinary appearance of a germinal tube, with double contour, and pale granular protoplasmic contents. The first branches are usually very irregular and stumpy, sometimes acquiring a knobby appearance, from the formation of one or two close-set septa; one of the ends, however, at length grows forth more directly, and rapidly produces a long, straight, or sinuous hypha, which becomes divided at pretty regular intervals by septa, like those of the first-formed tube, and which gives off branches to the right and left as it proceeds.

This more vigorous forward growth of the germinal hypha is preceded, in many cases, if not always, by the formation of a *haustorium*, from the point where one of the first-formed stumpy branches clings to the epidermis. This sucking organ pierces the subjacent cuticle and cell-wall (fig. 5) by a long, very slender, curved neck, which ends below in an ovoid, nearly uniform granular mass. All parts of this organ are hyaline and colourless, contrasting sharply with the brown hypha whence it springs.

The growing end of the hypha is pale and almost colourless, the thickening and carbonising of the cell-wall commencing a little behind this point; the diameter remains the same, however, up to the bluntly rounded apex. As the main germinal hypha proceeds to form the network of mycelium, a zig-zag course comes to predominate from the manner of branching. Some of the branches grow out—very commonly at an angle of about  $60^{\circ}$  (*cf.* fig. 2)—as main

twigs of the mycelium, others remain short and stumpy, as did the earliest ones, and from these, as before, *haustoria* descend into the epidermal cells of the host-plant (fig. 6). In this manner the network of mycelium becomes closely anchored, at pretty regular intervals, to the epidermis.

But, besides tracing these *haustoria* by examination *en face*, they, and their origin and course, may be rendered more evident in vertical sections of the leaf, especially when hardened and stained. Specimens are well shown in fig. 8, where the razor has passed nearly through the points of junction between *haustorium* and hypha. But, although the *haustoria* are especially produced by the stumpy branches, they are not confined to these, but may arise along the course of the thicker or thinner main branches, as shown in fig. 6.

Moreover, some of the stumpy side-branches do not produce *haustoria* at all, but become broken up into short more or less quadrate bodies, by splitting at the closely-set septa. These fragments of the mycelium appear to reproduce the fungus in a vegetative manner, by simply putting forth hyphæ from any point (fig. 7). By the above-described means, then, a more or less symmetrical, radiating mycelium is produced on the surface of the jasmine leaf, gathering strength as it grows and as new *haustoria* branches, and anastomoses become established, and presenting the appearance to the unaided eye of a delicate, dark coloured, cloudy spot.

When thus developed, the appearance of reproductive organs soon becomes evident. The rudiments of these arise as follows.

Here and there, at certain points in the network, one of the short stumpy lateral branches is noticed to form numerous lateral lobes, which remain closely appressed side by side, and gradually build up a flat, disc-like expansion (figs. 9 to 13). The successive lobes, which collectively form this disc, become cut off by closely-set septa as they become completed, and the formation of the whole strongly reminds one of the dichotomous mode of growth of some Algæ (*e.g.* the thallus of *Coleochaete*). This is still evident in an advanced disc, as fig. 13, which was very carefully drawn with special reference to the position of septa, &c. It will be understood that the *radial* walls in such a disc result from the coalescence of the out-growing lobes side by side; the *tangential* walls, on the contrary, are true septa.

While this disc is commencing to form, or in some cases (figs. 28—30) before the division and growth of the lobes

have taken place, peculiar tufts of very delicate, pale hyphæ arise from the mycelium at the points indicated.

The lateral lobes become pale and transparent, and split up into masses of fine filaments embedded in a common gelatinous matrix, whence spring the tufts referred to. In other cases, the disc is already formed before the tufts of delicate hyphæ appear, radiating from the edges, and embedded (during the wet season) in jelly as before. In the latter case, the tufts take their origin especially from the inner walls of the disc (figs. 20 and 26), resulting, apparently, from the splitting up of the cells into finer hyphæ. In other cases, the disc becomes completed, or nearly so, before the delicate hyphæ appear as a fringe from beneath its edges. What are these tufts of slender filaments embedded in mucilage?

From the extremities of the fine threads pale, curved, slender spore-like bodies (figs. 25—27) are abstricted. These bodies appear to be *sporidia*, though I have failed to make them germinate. From the close proximity of these tufts to rudimentary or advanced *perithecia*—for, as we shall see shortly, the disc becomes the protecting body to the ascus-bearing “fruit”—and from the above-named fact, it might be argued that the “*sporidia*” are really not spores but “*spermatia*,” and no doubt there is much to be said for such a view; but there are also facts which militate against this view. At present, on account of the difficulties in determining the exact connection of these very minute filaments with the larger hypha-branches, some doubt must be expressed as to their exact relations.

To return to the disc, which certainly arises by successive lobing and septation of the stumpy lateral branch, as described. When this has attained a certain size the centre becomes raised and a boss-like body results. This is caused by the pressure of an increasing mass of fine filaments beneath. In this dense internal mass arises the ascus-bearing tissue.

From the above it will be observed that the disc—a mass of dark-coloured, thick-walled cells compressed into a tissue—is a vegetative structure, and cannot be looked upon as the product of a sexual act. It is true in some examples the disc arises at a point where an anastomosis of hyphæ occurs (fig. 9), but it is also true that in the majority of cases the whole course of its formation has been traced as a simple development of the lateral lobe of the hyphæ. Unless we can regard the lateral compression of the first two outgrowths of this as a sexual act, it becomes certain



that the disc is an asexual formation; and such I interpret it to be. The task of determining what takes place beneath this disc has been the most difficult of all, not only on account of the extreme minuteness of the structures to be described, but also because their delicate nature, in contrast with the brittleness of the rest of the fungus and the leaf,<sup>1</sup> renders it very difficult to obtain the necessary sections. Partly from fresh specimens, and partly from preparations in alcohol and glycerine, however, the following facts are to hand.

Vertical sections through the disc, &c., when the first signs of its upheaval as a boss are apparent (figs. 14 and 15), show that the space between it and the epidermis of the leaf is becoming filled with closely packed, delicate hyaline hyphæ, forming a translucent meshwork, containing no air, and very difficult to examine in the fresh state. By dissection and observations of the inner walls, it becomes evident that these delicate filaments spring from branches developed from the inside of the disc (figs. 20—22); these converge, and form a mass of interwoven filaments in the cavity of the boss, the increase of which forces up the disc in a dome-like manner as described. It is in the substance of this dense feltwork that the asci and spores subsequently arise. In the meantime any of the larger hyphæ which happen to be encroached upon by the spreading disc above described, become gradually broken up into short joints, which retain their thin walls and pale colour (fig. 32). What becomes of these "toruloid" masses I cannot determine, and am of opinion that the phenomenon is of an accidental nature, and has no important bearing on what follows.

At a stage somewhat later than that depicted in fig. 15, one finds, from dissection of the boss or raised disc and its contents, that a series of delicate, shortly jointed, branched, and more or less coiled hyphæ have arisen in the substance of finer filamentous meshwork in the interior, and that certain short branches, or lateral cells, of these become densely filled with finely granular protoplasm, and rapidly swell up into globular and pyriform bodies (figs. 21 and 18): these are the young asci. Each consists of a delicate cell-wall enclosing the protoplasm. As the ascus increases in size the contents become divided (figs. 23 and 24) into four nucleus-like bodies, arranged in a tetrahedron in a mass of more coarsely granular protoplasm. At a later stage, each of the nucleus-like masses becomes divided again, and these

The leaf becomes crowded with large crystals, apparently of calcium-oxalate, slowly soluble without effervescence in hydrochloric acid.



bodies—eight in all—become the future ascospores. During the later stages most of the finer filaments disappear, apparently by absorption, and by this time no trace of the external tufts of fine hyphæ is left. In a vertical section of the nearly ripe fruit-body, the asci are seen to spring from an interwoven mass forming the floor of the domed chamber (figs. 18 and 22), and by the absorption of water and increase of pressure, the roof becomes split into radial, irregular segments at a later date (fig. 3), the structure giving way at the weakest parts—*i. e.* where the walls are merely coalescent side by side—hence the stellate character of the rupture as the asci burst irregularly and emit their ripe, brown spores.

We have thus far left undetermined the origin of the ascogenous curved hyphæ found in the mass of finer filaments forming the inner mass of the fruit body. In a vertical section of a boss, at a stage somewhat anterior to fig. 16, I have obtained a structure figured in fig. 17, and if we may accept the analogy with what occurs in *Erysiphe*, &c., it appears probable that this is the ascogonium or sexual organs of the fungus.

Within the mass of felted filaments derived from the inner walls of the domed disc, has arisen a subglobular mass of extremely thin-walled cells, which appear to be united into a solid ball of cells. The resemblance of this body to the young *perithecium* of certain *pyrenomyces* is too evident to be ignored. Nevertheless, I have not succeeded in tracing the details of its formation. That this body consists of the ascogenous hyphæ figured at figs. 19 and 21 there can be no reasonable doubt, but whether it arises after the action of spermatia on an ascogonium, or by the copulation of an antheridial branch with an ascogonium, must remain for the present undecided.

All things considered, it appears most probable that this coil of ascogenous hyphæ arises quite internally, and we have already seen that no evidence exists for believing the outer tissue of the fruit-body—the domed disc—to have arisen by a sexual process. If this be so, it would appear that the disc or dome of carbonised cells is not the true *perithecium*, but a sort of covering body, within which the true fruit-body arises. We have seen that the tufts of *sporidia*, or *spermatia*-bearing filaments, arise from and near this outer covering of cells, and analogy might suggest that, just as in many other *Ascomycetes* the homologous hyphæ spring from the *stroma*, so here we have a *stroma* fore-shadowed in the protective disc formed before the true sexual

organs (delicate as they are) become formed. It is not difficult to imagine how, in other *Ascomycetes*, an advantage was gained when the *stroma* became fused with the subjacent epidermis, or leaf tissues; and it seems clear that the deep shade afforded by the dense walled cells of the disc must be of benefit to the fungus, exposed as it is on the top of the leaf to a tropical sun. If this explanation is the true one, it becomes easier to understand the relations of this fungus, which I will now proceed to examine.

In the general characters of the mycelium and fruit bodies, as well as in its mode of life, this *Ascomycete* is clearly a *pyrenomycete* apparently allied to the *Meliolas*,<sup>1</sup> several species of which occur in Ceylon;<sup>2</sup> and from the details of structure of the *perithecium* and spores, it appears probable that it should be placed in the genus *Asterina*,<sup>3</sup> of which it may or may not be a new species.

Bornet (*loc. cit.*) had already pointed out the analogies between *Meliolæ* and the group of *Erysiphei*; and, indeed, it has been assumed that the latter group is represented by the former in the tropics.<sup>4</sup> The analogies are no less striking, in some respects, with the present fungus; and the discovery of haustoria, which only enter the outer cells of the host, render them still more important. If the structure figured at fig. 17 prove to be a definite *Ascogonium*, &c., formed on the type of *Erysiphe* itself, the analogy is rendered complete, and the "disc," or *stroma*, must be looked upon as something superadded.

As to the injury caused to the individual cells by the haustoria, no direct evidence has been obtained by the use of reagents, &c. No callus or hypertrophy of the invaded tissues can be detected, and it appears highly probable that the parasite only absorbs very soluble, probably largely mineral, contents of the cells. Even when the sickly yellow spots appear the cells do not show obvious injury: each becomes occupied by a single large crystal of calcic oxalate, which, however, appears sooner or later in old leaves not invested by the fungus. No doubt some injury results from the excessive shading, when the black mycelium is abundant on the upper surface; but there is no evidence to show that the parasitism ever becomes more intense than has been described.

<sup>1</sup> Cf. Bornet's paper in 'Ann. des Sc. Nat.,' tom. xvi, 1851.

<sup>2</sup> Cf. Berkeley and Brown, in 'Jour. Linn. Soc.,' vol. xiv.

<sup>3</sup> 'Bot. Antarctic Voyage,' and 'Journ. Linn. Soc.,' vol. xiv. Lévillé,  
'Ann. d. Sc. Nat.,' t. iii, p. 59.

<sup>4</sup> See also Berkeley, 'Introd. to Crypt. Bot.,' p. 275.

*The THREAD-CELLS and EPIDERMIS of MYXINE.* By J. E. BLOMFIELD, B.A. (With Plate XXX.)

THE observations here recorded were begun some years ago by the advice and with the supervision of Professor Lankester in the Botanical Gardens at Oxford, and by his kindness in supplying me with specimens and the use of the Zoological Laboratories of University College, London, I have been enabled to verify and extend them.

The epidermis of the various Cyclostomes has been the object of researches by several different observers, but the most exhaustive account is contained in a paper by M. Alexandre Fœttinger, which appeared in March, 1876, in the 'Bull. de l'Académie royale de Belgique,' 2me série, lx, No. 3. This thesis embodies the results of former writers, and gives a very complete account of the epidermis as seen in *Petromyzon fluviatilis* and *P. planeri* and the larval condition of Ammocœte. And since this description will serve us as a standard of comparison for the similar structures in Myxine, it will be necessary to give a short account of his researches before entering on the immediate subject of this paper.

The epidermis of *Petromyzon* is composed of a series of cells, of which M. Fœttinger recognises four chief varieties:

The ordinary epidermic cells.

The caliciform or goblet cells.

The club cells.

The granular cells.

The ordinary epidermic cells, arranged in series and pressing one against the other, form the major part of the epidermis. They possess a nucleus, and in the lower strata very often two nuclei. Their shape varies according to the situation in which they are found. In the deeper layers—that is, those next the dermis, they are prismatic or stalked, resting on an expanded base, and in the more superficial region the external series of cells has its free border thickened and perforated by fine pore canals.

The caliciform, or goblet cells, are similar to those found in other parts in other animals, as described by F. E. Schultze, and do not call for a lengthened description.

The club cells are much larger than the other elements of the epidermis, and are at once recognised by the yellow colour they assume after treatment with picrocarmine. They appear to consist of a membrane enclosing the yellow contents, which have a lamellated arrangement. At one pole of the oblong cell two nuclei are always present. As regards their origin and fate, M. Föttinger observed that they originated in the deep layer of the epidermis among the cells resting on the dermis, from which they were soon capable of being distinguished by their increasing size and tendency to yellow coloration with picric acid. As maturity is being reached, both these characters become more marked, and the cell itself slowly makes for the superficial layers of the epiderm. When it has reached this point it raises the superficial cells and is discharged to the exterior, where it may be seen resting, devoid of nucleus, on the superficial cells. As it progresses to the surface it leaves a finely granular mass behind to mark its course.

These bodies were supposed by former writers to be muscular (Max Schultze) or nervous (Kölliker) in nature; but, as M. Föttinger states, there can be little doubt that their function is glandular.

The granular cells are spherical or ovoid in shape, and lie embedded among the ordinary epidermic cells. Each possesses a nucleus surrounded by coarsely granular fluid material, which forms the cell substance, and the whole is enclosed by a membrane. From the side of the cell next the dermis several fine processes are seen, which dip down among the lower cells, and may reach the epidermis; in which case they terminate in an expanded base, similar to the epidermic cells found in this situation. Picrocarmine is the best reagent to distinguish them from the surrounding elements, as it stains them a bright red. They originate from ordinary epidermic cells in the deepest layer of the epidermis, and grow upwards, always remaining in contact with the dermis by their processes, till they reach the external layers of the epidermis, where they serve some purpose at which M. Föttinger will not hazard a guess.

#### *General Epiderm of Myxine.*

We will now pass on to the consideration of the epidermis in Myxine. On examining a section of the body-wall of a Myxine which has been stained with picrocarmine—for this



is the staining fluid which is most useful from its differential colouring of the various structures—there is seen (*cf.* fig. 1) most externally the epidermis, succeeded by the dermis, which in its turn rests on a peculiar subcutaneous connective tissue, much resembling the notochordal variety of that group in its general appearance, and these two latter structures merit a short account.

The subcutaneous tissue is rather remarkable. In a specimen which has been in spirit below the dermis there is seen an irregular network composed of spherical or hexagonal meshes, each mesh being composed of fibrous tissue continuous with and running into the dermis, and holding somewhere in its lumen a granular nucleus with nucleolus, which sometimes takes up a central at other times a parietal position (Pl. XXX, fig. 1 *c*).

If now a specimen be examined which has not been subjected to the action of spirit, but preserved in picric acid, this network is found to be filled with an oily yellow liquid, occupying the spaces in which the fibres and nuclei may be traced as before.

This tissue is to be regarded as adipose tissue, in which the primitive cells, supported in a framework of fibres from the dermis, have by a process of cell secretion become metamorphosed, leaving only the fibrous stroma and nuclei floating in the oil to testify to their former condition.

The dermis is a structure composed of layers of fibrous tissue, which take up the carmine of the picrocarmine and assume a bright pink colour. Here and there between the laminae may be seen pigmented cells (fig. 1 *b*).

The epidermis rests on the dermis (fig. 1 *a*), and is composed of several layers of cells, among which a difference may at once be recognised by the use of a staining fluid, such as borax carmine, which divides it into two portions, of stained and unstained matter, much in the same way that the epidermis of higher vertebrates is distinguished into the stratum mucosum and stratum corneum, the stained portion being composed of living protoplasmic cells and the unstained portion of formed cells, whose protoplasm has disappeared in the process of producing the particular body which in the case we are considering is mucus and in the higher vertebrates keratin.

That the substance is mucus in the case of *Myxine* is probable from the shape of the cells, which conform in character to the typical mucus or goblet cells found in the alimentary and respiratory tracts. These goblet cells are arranged in series two or three deep. They vary slightly

in shape, being sometimes pyriform, at others caliciform, but in all the shrivelled remains of the nucleus can be seen at the base. The cells of the most external row sometimes present a beaded appearance on their free surface, which reminds one of the external cells in *Petromyzon*, which have their side much thickened.

In the deeper parts of the unstained layer young goblet-cells may be seen, which possess a normally-sized nucleus, and are capable of staining; and between these and the mature mucus-cell all stages are recognisable—in fact, there are few tissues in the animal kingdom that afford such good examples of goblet cells and their formation (fig. 2*a*).

The ordinary epidermic cells need not detain us. The lowest series (fig. 2*b*) have an expanded base, and the higher form transitions to the mucus cells; but embedded among them are two larger kinds, which merit a longer description.

The first kind are stained yellow by the picric acid (fig. 1*d*). They occur at all parts of the epidermis, and may sometimes be seen protruding between the superficial cells at the surface. In shape they are irregular, but always oblong, appearing to have their form determined by the ordinary cells which surround and firmly embed them, for it is impossible in teased preparations to obtain them quite isolated. Their appearance is coarsely granular, and no obvious nucleus is present, but often a small red spot may be detected, which would appear to be the remains of that structure. From the fact that they stain yellow with the picric acid of the picrocarmine one is led to the idea that they correspond to the "club cells" of *Petromyzon* described above; and though they are not so complicated in their structure, yet their behaviour with picric acid and similarity of their fate—for, like those cells, they evidently grow towards the surface and are discharged—leave little room to doubt that they are homologous.

The second kind of cell (fig. 2*e*) alluded to above would seem to correspond to the granular cells of *Petromyzon*, though the correspondence is not so well made out. In most of my sections, which were prepared from specimens hardened in chromic acid or Müller's fluid, these cells appear as clear spherical spaces embedded among the ordinary epidermic cells which are compressed and flattened in their immediate neighbourhood.

The periphery is bounded by a membrane, and in the interior is a finely granular mass containing a deep-stained spot, which appears to be the remains of a nucleus. From the granular mass processes of similar substance radiate

towards the periphery, giving the whole the appearance of a spider.

In some specimens, however, which had been placed in alcohol without previous treatment with chromic acid, the appearance presented by these cells was different. For then they seemed to be filled with a coarsely granular material, exceedingly greedy of the carmine of the picrocarmine, so that they quickly became stained of a bright pink hue, in which it was impossible to make out the presence or absence of a nucleus (see fig. 8 *e*). This difference in the cells, according to their previous treatment, seems to show that they contain a liquid material, which is precipitated by alcohol, but not by chromic acid or Müller's fluid. If these cells correspond to the granular cells (*cellules granuleuses*) of *Petromyzon*, one would expect to find processes from them towards the dermis, but this I have not been able to do. As regards the function of these cells, I am in no better position than M. Föttinger to offer suggestions; but from the behaviour of similar cells in the glands, which will be considered in the sequel, it seems probable that they contribute some part to the mucus, possibly the more liquid portions of that secretion.

#### *The Lateral Glands of Myxine.*

*Myxine* is characterised by the large amount of mucus which it is capable of ejecting, so that it has earned the specific name of "*glutinosa*," a term, the appropriateness of which may be judged by the fact that two individuals thrown into a bucket of water are capable of gelatinising the whole with their secretion. This secretion comes from a series of glands which are placed on each side the body, and open by a series of pores, easily seen on the exterior. Each gland lies beneath the dermis, embedded in the subcutaneous tissue, and having the lateral bands of muscle on its inner side, which are pushed inwards by the distended gland, and appear capable of exercising an influence in ejecting the contents, though to what extent this is actually the case it is hard to say in the absence of direct experiment.

These glands are to be regarded as solid involutions of the integument in which the epidermic cells have acquired a more specialised structure in connection with the production of mucus. They are lined by a fibrous wall, which, in sections of a small and young gland, appears to be composed of several layers, though in the fully-developed organ the layers are fused into one. Resting on the wall and filling the gland are the peculiar yellow cells, the characteristic elements of



the secretion, supported by a stroma, and it is possible to trace the continuity of the cells in the gland with those of the epidermis through the stalk of the invagination, so that the fibrous wall is obviously continuous with the dermis, and the cell elements of the gland with those of the epidermis.

If the contents of a gland be squeezed out and received on a slide (*cf.* fig. 5) and examined, they are seen to be composed of a quantity of large oblong yellow cells, which appear to be unwinding, and giving origin to long coiled and twisted threads running in various directions across the field of the microscope. Besides these are others bearing a considerable resemblance to spiders, which appear to form a stroma for the support of the other cells. These "spider cells" are found in specimens preserved in spirit.

Of these two structures, the first, which we will designate "thread cells" (*cf.* fig. 6), are of particular interest, from the peculiarity of their construction. In shape they do not exhibit much variation. They are always oblong, sometimes larger at one end than the other, but generally more or less ovoid. They stain very readily with picric acid of a bright yellow hue, and under the action of carmine exhibit a nucleus or remains of such at one of the poles (the head). This, which in the unstained cell seems to be of the nature of a vacuole, after long treatment with staining fluids, is seen to be a nucleus, often containing one well marked and comparatively large nucleolus. On careful focussing when examining the mature cells it is seen that the yellow material is divided into threads, which are wound round a central core of granular matter. The exact mode in which the thread is wound is rather hard to determine, but it seems that most superficially it is arranged transversely to the long axis of the cell, while in the next layer it runs parallel with it (fig. 7). When the cell is mature and while it is in the gland, or after its discharge, it begins to unwind. This process begins at the pole opposite the nucleus, and it seems that the cell is unravelled by a single thread. These threads are found in the mucus at the surface of the body of the animal in association with epidermic and goblet cells.

In sections of a young gland (*cf.* fig. 8) where the elements are not mature, it is possible to trace the origin of the thread cells from the ordinary epidermic cells. As has been stated above, the glands are to be regarded as solid involutions of the epidermis, in which the various epidermic cells undergo a change in connection with the secretion of mucus, and these two elements—the "thread cells" and "spider cells"—may be seen to have their origin from ordi-



nary epidermic cells. After staining with picrocarmine, it is easy to trace the development of the thread cells, because as the yellow-staining material is deposited in the cell the red tint gives place to a yellowish red, and then becomes altogether replaced by yellow, except the nucleus and a small zone of granular matter around it, which retains its pink tint. As the deposition of the yellow matter progresses striæ make their appearance, which indicate the commencement of the thread-like arrangement that obtains in the mature cells (fig. 9).

It seems a matter for surprise that these curious cells should have hitherto escaped description, but as far as I am aware there is only one drawing of them, which occurs in Leydig's 'Manual of Histology' (French translation, p. 225), in which they are figured with threads appended to their tails and described as nerve cells, the thread being regarded as the fine termination of a nerve.

The second kind of cell, the "spider cell," would appear to represent the granular cells (Foettinger) of the epidermis, which, when enclosed in the glands, undergo a process whereby they lose their contents, and nothing is left save the membrane and nucleus, and a granular material round the latter. It may be that these cells are concerned in the nourishment of the thread cells, or, as seems more probable, they themselves contribute a constituent of the secretion, probably the more liquid part. When in the gland they form a sort of weak stroma, holding together the thread cells.

*The EYE of SPONDYLUS.* By SYDNEY J. HICKSON, B.Sc., B.A., Scholar of Downing College, Cambridge, Assistant to the Linacre Professor, Oxford.

THROUGH the kindness of Mr. Balfour I was enabled to study the structure of the eye of one species of this genus of Lamellibranchs. The margin of the mantle of several specimens was sent to him from the zoological station at Naples, treated with osmic acid and absolute alcohol, and with absolute alcohol alone. I was unable to determine the species to which the specimens belong.

The eyes of *Pecten* have been studied, as I pointed out in my last paper,<sup>1</sup> by several observers, but I have been unable to find any special reference either in figures or in writing to the eye of *Spondylus*. I hope, therefore, that this paper will supply the omission.

The eyes are situated on the edge of the mantle among the fringing tentacles, and seem to be as numerous as they are in *Pecten*, but one important difference is that the stalk which is so apparent in the latter genus is here very short, in fact, in some cases the eyes seem to rest on the edge of the mantle itself. Being considerably smaller in size than in the large *Pecten maximus* I have been unable to follow out all the details of the histology of the various parts, but I have observed all the main points in the general anatomy of the eye tolerably satisfactorily.

The cornea is composed of the same layers as in *Pecten*, the external epithelium of columnar cells being well marked. Round the edge of the cornea this epithelium becomes much thicker and densely filled with the dark brown pigment described in a similar position in the eye of *Pecten*, thus embracing the sides of the eye-cup.

The lens is, again, composed of nucleated cells which are more or less strap-shaped externally and internally become polygonal; but here the lens is large and projects into the cup of the eye as it does in *Pecten maximus*, leaving little or

<sup>1</sup> This Journal, Oct., 1880.

no space between it and the retina, such as is found in *P. opercularis* and, in a smaller degree, in *P. Jacobæus*.

The retina (*c*) stretches across the eye-cup as a broad band, leaving a considerable space behind for the tapetum and pigment, and but little space in front between it and the lens. The membrana limitans is not bent up in the middle as it is in *Pecten maximus*, but exhibits slight sinuation as it does in *P. opercularis*. Thus, in this genus we have combined the forward position of the retina with but slight sinuation of the membrana, a condition I have not found in any species of *Pecten*.



FIG. 1.—Diagram of the eye of *Spondylus*. *a*, cornea; *b*, lens; *c*, retina; *d*, tapetum; *e*, pigment; *f*, retinal nerve; *g*, complimentary nerve; *h*, epithelial cells filled with pigment; *k*, tentacles.

The distribution of the nerves is the same as it is in *Pecten*, both the retinal (*f*) and complimentary (*g*) branches being easily traced.

There is in this genus, too, a well-marked tapetum (*d*), composed of the same histological elements which I described in *Pecten*. There is nothing to be said about the pigment (*e*) further than that it is of the same brick-red colour as in *Pecten*, is apparently of the same granular composition, and occupies a similar position behind the tapetum.

As regards the morphological value of this eye, it is impossible at present to say anything definite, but it seems probable that the shortness of the stalk, the position of the retina, and the slight development of a vitreous cavity point to the fact that it is not as highly developed as the eye of *Pecten*. It probably presents a more complicated condition

than that of the eye-spots found in a similar position in certain other Lamellibranchs. This point, however, can only be settled by a further study of the anatomy of the eyes of these other forms, together with the development of them in those genera in which they are so well marked.



NOTE on OPEN COMMUNICATION *between the* CELLS *in the*  
PULVINUS *of* MIMOSA PUDICA. By WALTER GARDINER,  
B.A., late Scholar of Clare College, Cambridge.

AN anatomical investigation of the organs of motion in certain plants which I have undertaken at the suggestion of Professor Sachs has already led to some results which appear of sufficient interest for publication. A point of primary importance was to endeavour to ascertain whether any means of communication exists between the protoplasm of neighbouring cells. My attention has as yet been chiefly occupied with a study of the pulvini of *Mimosa*, *Robinia*, *Amicia*, and *Phaseolus*.

The parenchyma of the organs of motion in these plants in common, so far as I am aware, with that of all pulvini, is very strongly pitted, and as a result of my investigations it appears certain in the case of *Mimosa*, and most probably in the case of the other three, that by means of these pits there is a direct communication from cell to cell, or, in other words, that the pits are open. My method of preparation of the cells of the pulvinus, upon which these results in a great measure depend, is a modification of that first used by Sachs for the demonstration of sieve-pores, the only difference being that aniline colours instead of iodine are used as the staining reagent. The best results were obtained with thin sections of the fresh material. On treating such a section first with strong sulphuric acid and then with the aniline colour, or with a mixture of strong sulphuric acid and aniline colour, the middle lamellæ of the cells remain as a fine network, the cellular cell-walls are dissolved or rendered invisible, and the protoplasm presents the appearance of a deeply stained, irregularly shaped mass lying in the swollen colourless substance of the cell-wall.

From these irregularly-shaped masses radiate numerous fine processes from the central mass to the middle lamella. In any two neighbouring cells the processes from the one central mass meet exactly those proceeding from the other,

presenting a most characteristic appearance. From a very careful examination of numerous sections it appears to me that in a number of well-defined instances the protoplasmic threads are optically continuous. In other cases union between the contents of corresponding pits appears to be affected by a sieve-plate-like arrangement. Some of the processes on the other hand appear to end blindly, and do not reach as far as the middle lamella. When the protoplasm has undergone considerable shrinking and a certain tension has thus been brought to bear upon the threads, rupture of the latter frequently occurs, which rupture, however, seldom takes place at the point where the threads cross the middle lamella, but nearly always on one or on both sides of this point. These results appear to point out clearly the intimate connection existing between cell and cell. I am unable to speak with the same certainty on the subject of *Robinia*, *Phaseolus*, and *Amicia*, although the results hitherto obtained are of the most promising description. I hope shortly to be able to publish a more detailed account of the whole subject, together with an investigation of the stamens of the *Cynaraceæ* and of tendrils, in which latter some indications of the same structure have been observed.

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NOTES on the DEVELOPMENT of MOLLUSCA. By ALFRED C. HADDON, M.A., Professor of Zoology, Royal College of Science, Dublin. (With Plate XXXI.)

WITH the exception of the investigation on *Purpura*, all the following notes were made in the spring of 1879, while I was occupying the table belonging to the University of Cambridge in Dr. Dohrn's Zoological Station at Naples. I would here express my thanks to the University, and, at the same time, to the officials of the Zoological Station for their uniform kindness to me.

The following notes are purposely fragmentary, as I do not wish to lengthen my paper by needless repetitions of other people's work. It is, however, but fair to myself to state that I have made an immense number of observations, preparations, and drawings, more or less of which corroborate the researches of the numerous previous investigators of Molluscan Embryology.

*Nudibranchiata*.—The eggs of *Elysia viridis* are extremely favorable for showing the earliest developmental phases, such as the male and female pronuclei (impregnation), constriction of the polar cells and the concurrent well-marked amoeboid movements of the egg, radial striæ and other phenomena of segmentation, all of which can be readily studied in the living ovum.

In the majority of the Nudibranchs the segmentation is very regular, resulting in the formation of a well-marked gastrula, which is usually formed by a kind of rolling over combined with invagination. The extreme of the former process of gastrula formation is apparently reached in the Nematoid *Cucullaneus*.<sup>1</sup> The slit-like blastopore closes over from behind forwards, and I have reason to believe that in *Piona* (sp.?) at least the blastopore either becomes the permanent mouth, or the latter is invaginated at the spot where the former finally closes up.

At the commencement of the Veliger-stage two large cilia make their appearance in the centre of the velar area.<sup>2</sup> There

<sup>1</sup> Bütschli, 'Zeit. f. Wiss. Zool.,' xxvi, 1876.

<sup>2</sup> An anticipatory notice of this and of the succeeding observation will

can be no doubt that these cilia, which, owing to the difficulties of observation, have escaped previous investigators, are homologous with the cilia or ciliated tuft found in a similar spot of the pre-oral lobe in some Lamellibranchiata,<sup>1</sup> Dentalium,<sup>2</sup> Chiton,<sup>3</sup> Heteropoda<sup>4</sup> and Pteropoda,<sup>5</sup> and which is of such constant occurrence amongst the Vermes. These cilia are retained till the velum itself is lost. I have observed them in *Fiona*, *Polycera quadrilineata*, *Elysia*, and *Philina aperta*, and have no doubt that they are characteristic of the whole group.

The character of the groove at the edge of the velum and its relation to the mouth is well shown in figs. 4 and 5. The groove is clothed with fine cilia and the lower border has a fringe of larger cilia, but these are not nearly so large as the powerful motor cilia of the upper border. We thus have a pre- and a post-oral circle of cilia. This groove has been described and figured by Fol as occurring in Pteropods and Heteropods, and is indicated by Lankester in a sketch of the veliger of *Polycera quadrilineata*, though not described by him.<sup>6</sup> Fol ascribes a nutritive function to this groove. Though I carefully looked for food particles passing along it, I was disappointed, but I have no doubt that such is the case. Balfour further alludes to it in vol. ii, p. 306, of his 'Treatise.' The velar groove is probably common to all the Nudibranchs.

In all the Gasteropoda I have examined I have found a patch of cilia either round the anus, or at that spot where the anus will appear.

I have often seen, in optical section, thickenings of the epiblast in the regions of the supra-oesophageal and pedal ganglia, and have no doubt that they were the rudiments of the nervous system, but this can only be satisfactorily demonstrated by means of sections. The sense organs, too, have an epiblastic origin.

*Prosobranchiata*.—The prevalence of westerly storms when I was at Naples gave me an opportunity of examining *Janthina fragilis*. The ovum, which is crowded with coarse yolk spherules, divides into four segmentation spheres in the ordinary manner. Fig. 6 shows the next stage, the four new epiblast

be found in vol. i, p. 189, of the late Prof. Balfour's 'Treatise on Comparative Embryology.' I cannot refrain from here acknowledging my great indebtedness to my late beloved master and friend.

<sup>1</sup> S. L. Lovén, "Vetensk. Akad. Handl.," 1848, translated in 'Arch. f. Nat.,' xv, 1849.

<sup>2</sup> H. de Lacaze Duthiers, 'Ann. d. Sci. Nat.,' 4th ser., vii, 1857.

<sup>3</sup> S. L. Lovén, 'Öfversigt Vetensk. Akad. Förhandl.,' 1844.

<sup>4</sup> H. Fol, 'Arch. d. Zool. exp. et gén.,' v, 1876.

<sup>5</sup> Ibid., iv, 1875.

<sup>6</sup> E. Ray Lankester, 'Phil. Trans.,' 1875 (pl. x, fig. 8).



cells being much smaller in size, and are formed of clear protoplasm. I am inclined to think that the next stage is formed by other four cells being segmented off from the four large yolk cells (fig. 8) ; be this as it may an epiblastic cap is soon formed, which spreads over the yolk cells. Fig. 10 shows that at the usual position, at the tip of the blastopore, mesoderm cells (*m*) are segmented off from the yolk cells.

The further development of *Janthina* presents us with nothing which is not common to most other Prosobranchs. I would, however, draw attention to the large violet mass which is seen on the right side of the embryo close to and dorsal to the anus. A similar pigmented mass is met with in many Opithobranchs, large and red on the right side in *Philine aperta*, a small violet spot on the left side of *Elysia virides* ; in *Pleurobranchidium*, as first noticed by Lankester (loc. cit.), there is one on each side coloured pink. It would be interesting to discover the meaning of this pigment.

It is well known that the nervous system arises from the epiblast throughout the animal kingdom, with the exception of certain nerve elements in the Cœlenterata, which have a hypoblastic origin, and of the central nervous system in Prosobranch, Gasteropoda, and in Cephalopoda, which is stated to arise from the mesoblast. It is quite comprehensible how the former exception to the general rule should arise, but the two latter are, to say the least of it, very anomalous ; and it was for the purpose of attempting to clear up the difficulty that the following researches were made.

In all the groups of the Gasteropoda, with the exception above noted, the origin of the nervous system from the epiblast has been observed with more or less accuracy. I shall now describe the manner in which the central nervous system develops in two genera of the Prosobranchiata.

In *Purpura lapillus*, at the stage represented in fig. 12, two large thickenings of the transparent skin are seen with the velum in close connection with the developing eyes and incipient tentacles, and two similar masses occur in the foot, having an intimate relationship with the otocysts. A transverse section through this region shows us that the nervous system is in process of development by proliferation from these paired thickenings of the epiblast. Fig. 14 gives a more highly magnified view of one of the supra-œsophageal ganglia thus being formed. It is worth while to compare this figure with that given by Rabl of *Planorbis*.<sup>1</sup> From observations made by transverse sections I have no hesitation in expressing my conviction that a precisely similar origin of the nervous system occurs

<sup>1</sup> C. Rabl, 'Morph. Jahrb.,' v, 1879 (Taf. xxxv, fig. 20).

in *Murex erinaceus*. Fig. 15 shows the proliferation (*pg*) which forms a pedal ganglion. Close by it is seen the newly-formed otocyst (*ot*). The sense organs are developed by involution from the same tissue (see figs. 16 and 17).

These statements differ most materially from those of Bobretzky,<sup>1</sup> who "plodded at section-cutting to elucidate the question as to the origin of the nerve elements," and who arrived at the conclusion "that (in *Fusus*) the ganglia arise as a massing together of mesoderm cells" (p. 143). I would venture to suggest that we may regard his account as either depending upon the occurrence in *Fusus* of "precocious segregation" (Lankester), as is apparently the case in the Cephalopoda, or, more probably, arising from an error of observation on the part of this accurate and painstaking observer; for it seems to be very unlikely that *Fusus* should differ so much in the development of these important organs from the allied genera *Purpura* and *Murex*.

According to Fol,<sup>2</sup> in *Limax*, the pedal ganglia arise from the mesoblast of the foot, while the supra-oesophageal ganglia are developed from the epiblast of the velum; this manifestly requires corroboration.

Accepting these conclusions then, the Cephalopoda appear to be unique in having a mesoblastic nervous system—a point which requires to be again worked over.

<sup>1</sup> N. Bobretzky, 'Arch. f. Mik. Anat.,' xiii, 1876.

<sup>2</sup> H. Fol, 'Compt. Rend.,' 1875, p. 523-6.

NOTES on ECHINODERM MORPHOLOGY, No. V.<sup>1</sup> *On the HOMOLOGIES of the APICAL SYSTEM, with some REMARKS upon the BLOOD-VESSELS.* By P. HERBERT CARPENTER, M.A., Assistant Master at Eton College.

DURING the last two years the morphology of the Echinoderms has engaged the attention of several continental naturalists. Some of their observations tend to support views which I have advanced in the pages of this Journal, while others are directly opposed to them. Under the latter head come the researches of the French school into the anatomical relations of the vascular system; and it is curious that the conclusions to which Messrs. Jourdain, Koehler, Apostolidès, Perrier, and Poirier have been led differ *in toto* from those of the German school as represented by Greeff, Hoffmann, Lange, Simroth, Teuscher, and especially by Ludwig. These last have been sufficiently described in a previous number of this Journal (vol. xxi). It is hardly time as yet to give a *résumé* of the observations of the French school, but one or two of their principal conclusions may be briefly alluded to.

Both Perrier and Apostolidès have published figures of their preparations, the former having worked at the Urchins, and the latter at the Ophiurids; but neither Jourdain nor Koehler have as yet given more than brief abstracts of their results,<sup>2</sup> while the complete memoir of Messrs. Perrier and Poirier on the anatomy of the Asterids is not yet in print. All these observers, however, agree in one important point. The so-called "heart" or "central plexus" of an Urchin, Starfish, or Ophiurid is not a

<sup>1</sup> Under this title I propose to continue from time to time the series of papers bearing on this subject, which I have already published in previous volumes of this Journal, *e.g.* :

I.—'On the Oral and Apical Systems of the Echinoderms,' part 1, vol. xviii, 1878, p. 351. II.—The same, part 2, vol. xix, 1879, p. 176. III.—'Some Disputed Points in Echinoderm Morphology,' vol. xx, 1880, p. 321. IV.—'The Minute Anatomy of the Brachiate Echinoderms,' vol. xxi, 1881, p. 169.

<sup>2</sup> 'Comptes Rendus,' t. lxxv, pp. 1002-1004; t. xciii, pp. 651-653; t. xciv, pp. 139-141, and pp. 744-746; t. xcvi, pp. 459-461. See also Perrier and Poirier, in t. xciv, pp. 658-660, and pp. 891, 892.

part of the blood-vascular system at all, but an excretory gland. It is said (though not by Jourdain) to communicate with the exterior through the madreporite, instead of joining an aboral ring, as described by Ludwig; while it has no connection with any blood-vascular ring situated between the nervous and water-vascular rings. Neither are there any radial blood-vessels other than the subdivisions of the body cavity, which have been variously termed "nerve-vessels" or "perihæmal canals," &c.

Ludwig has not yet published any of his observations on the anatomy of the Urchins; and it will be interesting to compare his results with those of Perrier and Koehler. In the case of the Ophiurids, however, we have the elaborate work of Apostolidès<sup>1</sup> to contrast with those of his German predecessors. He was fortunate in having had an abundant supply of living specimens at his disposal, while Ludwig dealt exclusively with spirit material; but the differences between their results are too great to be explained by this circumstance alone.

The so-called "Herz" or "Herzgeflecht" of the Ophiurids is termed the "piriform gland" by Apostolidès; and he says (p. 147) that after the removal of the common envelope of this organ and of the water-tube, "Il est impossible de ne pas voir la communication directe de la glande piriforme avec l'extérieur." He gives a good figure illustrating this point, which it is interesting to compare with that given by Perrier<sup>2</sup> of the internal relations of the madreporite in an Urchin; and like Perrier, he finds his observations to be confirmed by the results of injections. Both authors assert positively that the other end of the piriform gland is entirely free, no vessel proceeding from it to join an oral ring. Both, and also Koehler, describe it as having a central cavity with a series of radiating cellular columns or *acini* disposed around it, points which can only be made out in fresh specimens.

In the face of these detailed observations made on fresh material it is difficult to avoid the suspicion that the so-called central plexus of the Echinozoa may after all be of a glandular nature, and unconnected with the vascular system, though on the other hand, it is quite possible that its connection with an oral ring may have been overlooked by the French naturalists. Many of their observations, and especially those of Jourdain, confirm the statements of Ludwig, which are, however, totally ignored by the French author.

<sup>1</sup> "Anatomie et Développement des Ophiures," 'Arch. de Zool. exp. et générale,' vol. x, pp. 121-224.

<sup>2</sup> "Recherches sur l'Appareil circulatoire des Oursins," 'Arch. de Zool. exp. et générale,' vol. iv, pl. xxiii, fig. 1.



According to Messrs. Perrier and Poirier,<sup>1</sup> "Ce que l'on persiste encore à désigner, dans nombre d'ouvrages, comme le cœur chez les Échinodermes, n'est donc, chez tous les animaux de cet embranchement, qu'un simple corps glandulaire." I shall be greatly interested in learning the results to which these authors are led by their investigation of the central plexus of the Crinoids. I have studied its connection with the intervisceral blood-vessels in *Antedon*, *Actinometra*, *Bathycrinus*, and *Hyo-crinus*. Its connection with the chambered organ below and with the oral blood-vascular ring above are not difficult to make out, and though its walls are glandular it has no communication with the exterior. Should the views of the French school respecting the "piriform gland" of the Echinozoa be really correct, there must be more difference between these animals and the Crinoids than we have suspected of late years.

With respect to the radial blood-vessels of the Ophiurids, however, I have little doubt that Apostolidès is entirely in the wrong, and that he has altogether failed to see them, although they have been figured by Simroth, Lange, Teuscher, and Ludwig in longitudinal and transverse sections of the arms of seven different species.<sup>2</sup> Apostolidès has partially confounded the radial blood-vessel with the perihæmal space above it (nerve-vessel, *auct*), although he was acquainted with Ludwig's works, in which the two are clearly distinguished. He redescribes this last under the name of the "radial space," and considers it as a part of a vascular system, "composé d'une série de lacunes, existant entre les différents organes, système complètement clos et ne communiquant nullement avec l'extérieur." In reality, however, the whole of this system is the reduced body cavity subdivided into various portions, somewhat as it is in the arms of the Crinoids; and the small radial blood-vessel situated immediately on the dorsal side of the nerve has been overlooked or misinterpreted by Apostolidès, who attempts to show that the German authors have confounded it with the water-vessel, though the two are separated by the whole height of the perihæmal canal, radial space, or nerve-vessel.

Thus, then, I cannot but regard the existence of radial blood-vessels in Ophiurids as a well-established fact, and they must certainly be connected by an oral ring, as are the water-vessels

<sup>1</sup> "Sur l'Appareil circulatoire des Étoiles de mer," 'Comptes Rendus,' March 6th, 1882, t. xciv, pp. 658-660.

<sup>2</sup> Judging from the meagre descriptions without figures, which have been published by Jourdain, Messrs. Perrier and Poirier, I strongly suspect that the real blood-vessels which have been seen and drawn by their German colleagues have escaped their notice.

and nerves. This is figured by Ludwig,<sup>1</sup> but Apostolidès has failed to find it, just as one often does in the Crinoids unless the sections happen to lie in one particular plane; and on p. 168 he gets rather confused about the aboral ring described by Ludwig. He considers himself to have established “que les prétendus vaisseaux formant le *cercle aboral* sont dus au tissu conjonctif, dont les granules se colorent très vivement;” but he goes on to ask: “Est il (Ludwig) sûr que son second cercle aboral n’est pas la série de bandelettes musculaires qui relient les vésicules de Poli aux intervalles interbrachiaux? Leur arrangement coïncide exactement avec celui du cercle aboral.” These muscular bands, however, would hardly have the structure represented by Ludwig on fig. 15, viz. a cellular (genital) cord in the centre, with the actual blood space around it, outside which is the perihæmal canal of this portion of the vascular system.

Passing now to another part of the subject, I would direct attention to the elaborate account of the development of *Asterina gibbosa*, which has been recently published by Ludwig.<sup>2</sup> It is accompanied by eight plates, which are filled with figures representing sections of the larva in many different planes, and it throws much light on many obscure points in the development both of Starfishes and of other Echinoderms, as will be seen in the course of the following pages.

In the face of the controversy relating to the central plexus of the Asterids it is interesting to learn something of its development and of that of the oral ring, with which Ludwig believes it to be connected in the adult. The latter commences as a cleft in the mesoblast beneath the slight protrusion of the left side of the larval gut, which develops into the fore-gut of the adult. This cleft ends blindly in front, but is continued upwards and backwards into a space which runs close by the water-tube, on its right as seen from the dorsal side. It then bends to enter the mesentery, which separates the cavities of the two peritoneal cæca behind the gut, and ends below at the position of the blastopore. This space, terminating blindly in the mesentery, is the rudiment of the central plexus of the adult. Ludwig says nothing more respecting its later development; but as the two ends of the mesentery, the one near the water-pore and the other near the anus, are eventually brought close together, it would seem probable that the part of the central plexus which it contains is that which joins the aboral ring of the adult, whence (according to Ludwig) the genital vessels arise.

Now in the Crinoid, the lower end of the mesentery separating

<sup>1</sup> ‘Zeitschr. f. Wiss. Zool.’ Bd. xxxiv, pl. xv, fig. 16.

<sup>2</sup> Ibid., Bd. xxxvii, pp. 1-98.

the two peritoneal cæca is just in front of the closed blastopore, between it and the position of the permanent mouth; and it is not improbable that it contains a blood-vascular space like that described by Ludwig in the Asterid larva. The blastopore marks the position of the anus of the Crinoid, and it is just between the mouth and anus of *Actinometra* that the labial plexus is chiefly developed, in which the genital vessels originate.<sup>1</sup> This labial plexus is really circumoral, concentric with the oral blood-vascular ring, but farther from the gullet; and the above considerations tend to support the suggestion which I ventured to put forward last year respecting the homology of this plexus with the aboral ring described by Ludwig in Starfishes and Ophiurids. If this be the case the central plexus of the Asterid is much shortened in the Crinoid, but expanded laterally instead. For the labial plexus and oral ring are in communication all round the gullet. Future observations on the development of the blood-vascular system of the Crinoids may, perhaps, show that there is primitively only one connection between the two, and that on the right of the primary water-tube, *i.e.* in the direction of the anus, between which and the mouth the labial plexus and its connection with the oral ring are chiefly developed in the adult.

Hence in the Crinoid the aboral blood-vascular ring would be on the ventral side, while it is dorsal in the Asterids. It is very curious that, according to Ludwig's researches on the Ophiurids, the aboral ring (*pace* Apostolidès) actually is on the ventral side of the disc in the interradian areas; while on the right of the water-tube there is a shortened central plexus connecting it with the oral ring; and, lastly, that the aboral ring contains a cellular genital cord, like that within the genital vessels which originate in the labial plexus of the Crinoids.

The observations of Jourdain<sup>2</sup> and Greeff<sup>3</sup> possibly indicate the existence of the same structure within the aboral ring of the Asterids. In the relations of the blood-vascular system, therefore, as in so many other respects, the Ophiurids seem to stand midway between the Asterids and the Crinoids.

Four years ago<sup>4</sup> I ventured to suggest some modifications in the current views respecting the homologies between the calyx plates of a Crinoid and those in the apical system of an Urchin; and at the same time I endeavoured to replace the irregular and empirical nomenclature then in use for the plates of the Crinoidal calyx by one of a more rational kind. By this I mean one which

<sup>1</sup> This Journal, vol. xxi, 1881, pp. 184, 185, Pl. xii, fig. 14.

<sup>2</sup> 'Marburg Sitzungsberichte,' 1872, No. 11, p. 164.

<sup>3</sup> 'Comptes Rendus,' t. xciv, pp. 744-746.

<sup>4</sup> This Journal, vol. xviii, 1878, pp. 352-383.



should be based on facts that are of primary importance in Echinoderm morphology, viz. the radiate arrangement of the vascular system.

Most of the leading writers on the Crinoids have adopted this idea, *e.g.* Messrs. Wachsmuth and Springer and Professor Wetherby in America; the distinguished Swiss palæontologist, Mons. P. de Loriol; Professor M. Neumayr in Vienna; Dr. Chr. Lütken in Copenhagen; and (with a slight modification) Professor Zittel of Munich. The principle of it is that the true *basal* plates are those which are interradiial in position, and are situated immediately beneath the radials. Should there be a ring of radially situated plates separating these basals from the top stem-joint, as in *Encrinurus*, *Extracrinurus*, *Marsupites*, and many Palæocrinoids, they are to be considered as an additional element in the calyx, and are best termed under-basals or *Infrabasalia*.

The homology of the radials of a Crinoid with the oculars of an Urchin is now universally admitted, and the plates beneath them, the basals proper (subradials or parabasals of the old nomenclature), are almost as universally regarded as homologous with the genital plates of the Urchins. This view, however, has recently been controverted by an authority which carries much weight in all questions relating to Echinoderm morphology, viz. that of Ludwig.<sup>1</sup> The genital plates of an Urchin or Asterid are regarded by him as equivalent to the orals of a Crinoid or Ophiurid. I have already endeavoured to point out some of the inconsistencies resulting from this mode of looking at things.<sup>2</sup> But although Ludwig has since published two papers bearing on this subject, he has not attempted to meet my arguments. He is, however, less dogmatic than before. We do not hear anything more about *an undoubted homology*, but he simply refers to the parallel he has drawn as being "meiner Ansicht nach;" and some of the observations recorded in these later papers seem to me to strengthen my position very considerably, as I shall now endeavour to show.

It will be remembered that in immediate contact with the dorsocentral plate of *Marsupites*<sup>3</sup> are the five under-basals,

<sup>1</sup> "Ueber den primären Steincanal der Crinoideen, nebst vergleichend Anatomischen Bemerkungen über die Echinodermen überhaupt," 'Zeit. f. Wiss. Zool,' Bd. xxxiv, pp. 318-332.

<sup>2</sup> This Journal, vol. xx, 1880, pp. 322-329.

<sup>3</sup> This Journal, vol. xviii, 1878, p. 358, fig. 3. I think it will be advantageous to limit the name "dorsocentral" to the central plate in the apical system of the Echinoderms, instead of using it as equivalent to the centrodorsal of the *Comatulæ*. This is another structure altogether, being merely the modified top stem-joint.



radial in position; then the interradial basals, and then the radials. Outside and alternating with these must have come the orals, one of them originally perforated by the primary water-pore.

The homology of the dorsocentral plate with the subanal plate of the Urchins was, I believe, first noticed by Lovén,<sup>1</sup> and so far as I know no one, with the important exception of Ludwig, has denied it.

There have, however, been differences of opinion respecting the homology of this dorsocentral plate in the other Crinoids. Agassiz,<sup>2</sup> Lovén, and more recently also Wachsmuth,<sup>3</sup> regard it as representing the centrodorsal of the monocyclic *Comatulæ* and the under-basals of the pedunculate Crinoids which have a dicyclic base. They do not, however, give it a homologue in *Pentacrinus*, *Platycrinus*, and the numerous other Crinoids in which the basals (= genitals) rest directly upon the top stem-joint, and there are no under-basals. Wachsmuth gives an instance of the metamorphosis of the five basals of *Edriocrinus* into one plate during the life of the individual as "a calcareous deposit is secreted around the base, which covers and obliterates the sutures between the plates." Much the same thing happens with the under-basals of *Agassizocrinus*, so that in most of the adult specimens "not even a vestige of the sutures formerly existing between the plates can be detected," and this "actual metamorphosis—during the life of the individual—of five plates into one seems to us to be strongly confirmatory of the views of Agassiz and Lovén."

I cannot admit, however, that the obliteration of the sutures between the five under-basals of *Agassizocrinus* is any proof of the collective homology of these plates with the primitively single dorsocentral of an Urchin; and Wachsmuth's attempt to support this position by instancing the fusion of the *basals* in *Edriocrinus* is hardly satisfactory. A similar obliteration of the inter-basal sutures occurs in *Holopus*, *Rhizocrinus*, and *Bathycrinus*, but Wachsmuth would hardly be prepared to say that the basals of these Crinoids are therefore homologous with the dorsocentral of an Urchin; and the logical result of his argument would be that the basals of *Edriocrinus* are homologous with the under-basals of *Agassizocrinus*, which I am confident he would never think of asserting.

For my own part, I have already pointed out<sup>4</sup> that there

<sup>1</sup> "Études sur les Echinoïdées," 'Kongl. Svenska Vetenskaps Akademiens Handlingar,' Bd. ii, No. 7, p. 65, *sqq.*

<sup>2</sup> 'Embryology of the Starfish,' pp. 62, 63.

<sup>3</sup> 'Revision of the Palæocrinoidæ,' part i, Philadelphia, 1879, p. 21.

<sup>4</sup> This Journal, vol. xviii, 1878, pp. 371, 372.

cannot be a true homology between a single plate developed in the centre of the abactinal system and a group of five others disposed in a ring around that centre; nor can I admit any homology between either the dorsocentral plate or the underbasals and a cirrus-bearing top stem-joint, viz. the centrodorsal of *Comatula*, as supposed by Agassiz, Lovén, Claus, Neumayr, and Wachsmuth. According to the best observations<sup>1</sup> the centrodorsal is developed in a ring which encloses the right peritoneal tube near its proximal end,<sup>2</sup> while the dorsocentral of the Echinozoa occupies the middle of its distal end; and since there is a plate developed in the embryo Crinoid in this very position, viz. the terminal plate at the base of the stem (fig. III, 1), I have been led to regard it as homologous with the dorsocentral of the Echinozoa, a view which I have been glad to find adopted by my friend Dr. Lütken.<sup>3</sup> An additional fact in its favour is as follows:—The dorsocentral of the Asterid larva appears in the immediate neighbourhood of the blastopore,<sup>4</sup> and it is only removed from this position in the Crinoid larva by the few delicate rings of limestone which represent the rudimentary stem-joints. When it first appears it is doubtless even closer to the blastopore, but we have no information respecting its very earliest development.

I have therefore seen no reason to modify any of the opinions which I published four years ago respecting the homologies of the Crinoidal calyx in the other Echinoderms. At that time I knew of no instance among the Echinozoa of a proximal ring of radially situated plates around the dorsocentral, and I was therefore led to suppose that the underbasals of *Marsupites*, *Encrinurus*, *Extracrinurus*, and many Palæocrinoids are unrepresented in the other Echinoderms. Now, however, I believe them to have been discovered by Ludwig in the young *Amphiura squamata*, together with representatives of the Crinoidal basals, which gives me another argument against his views respecting the homology of the Ophiurids and Asterids.

Ludwig's study of the development of the Ophiurid skeleton<sup>5</sup> has thrown a curious light on the observations of some of his

<sup>1</sup> *E.g.*, those of Sir Wym. Thomson, Dr. Carpenter, and M. Sars.

<sup>2</sup> Götte's attempt to disprove the annular origin of the centrodorsal is not a satisfactory one. Even supposing that he is right, and that the centrodorsal does originate out of five primitively separate elements, it cannot, on the views advocated above, represent the primitively simple dorsocentral of the Echinozoa.

<sup>3</sup> Dyreriget, 'En Haand. og Laerebog til brug ved højere Laereanstalter,' Kjøbenhavn, 1882, p. 597.

<sup>4</sup> 'Zeitschr. f. wiss. Zool.,' Bd. xxxvii, p. 60.

<sup>5</sup> 'Zur Entwicklungsgeschichte des Ophiurenskelettes,' 'Zeitsch. f. wiss. Zool.,' Bd. xxxvi, pp. 181-200, Taf. x, xi.

predecessors respecting the abactinal system. In the Ophiurids, as in the Asterids, there is a dorsocentral plate (figs. I, IV), around which are the five terminal plates of the future arms

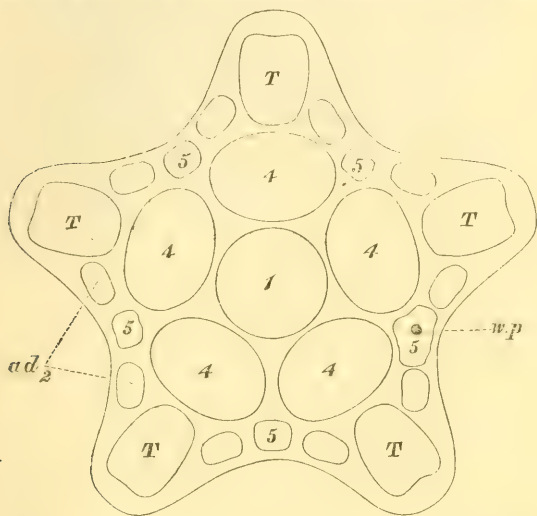


FIG. I.—Apical system of a young Ophiurid (*Amphiura squamata*), after Ludwig. The numbering of the apical plates is the same in this and subsequent figures as in the figures to my first paper (this Journal, vol. xviii). 1. Dorsocentral. 2. Primary radials. 3. Basals or genitals. 4. Radials. 5. Orals. T. Terminalia. *ad*<sub>2</sub>. Second adambulacral plates. *w.p.* Water-pore.

(fig. I, T; fig. IV, 4). As the rays grow these terminal plates are carried outwards from the disc by the development of new plates between them and it. In the young Asterid they are separated from the dorsocentral by the ring of rudimentary genital plates (fig. IV, 3), and eventually by other intermediate plates (*im*). But in the early Ophiurids the plates directly between the terminalia and the dorsocentral are radial in position (fig. I, 4), and not interrarial, as the genitals are in the Starfish. Hence, while the two rows of plates around the dorsocentral of the young Asterid are alternate in position (fig. IV), those surrounding the corresponding plate in the young Ophiurid are both radially situated, so that there are two plates in the direction of each ray, an inner and an outer one (fig. I, 4, T).<sup>1</sup>

<sup>1</sup> The presence of the orals (5) in the stage represented in Fig. I somewhat obscures this arrangement. It is better shown in the earlier stage represented in Ludwig's fig. 17, where the only oral visible is that which ultimately bears the madreporite.

According to Ludwig, this inner ring has been seen in *Amphiura squamata* and in other species (though not understood) by Schultze, Krohn, Agassiz, and Metschnikoff. He speaks of the plates as primary radials, from which I infer that he regards them as representing the radials of a Crinoid (fig. III, 4), and the ocular plates of an Urchin, though he never expressly says so. Together with the dorsocentral they form the rosette of six primary plates, which is a prominent feature on the abactinal surface of many adult Ophiurids. In *Amphiura squamata*, however, they become separated by the appearance of two rings of intermediate plates between them and the dorsocentral. The plates in the outer ring (fig. II, 3) are interradial, while those of

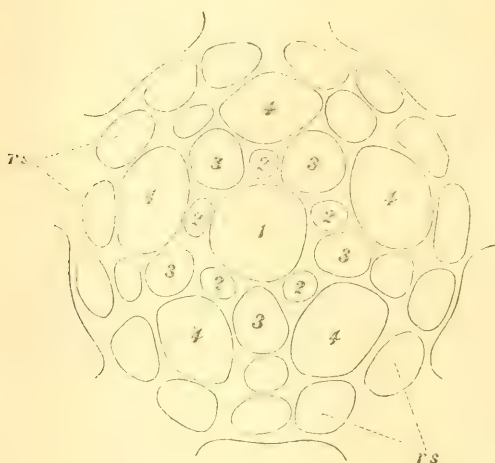


FIG. II.—Apical system of a slightly older *Amphiura*, in which the *Terminalia* have been carried out to the ends of the rudimentary arms, and the orals have passed over on to the ventral side. 1 and 4 as in Fig. I, after Ludwig. 2. Proximal row of intermediate plates representing the under-basals of a dicyclic Crinoid. 3. Second row of intermediate plates representing the basals of a Crinoid. Outside each of these are two other interradial plates. *r.s.* Radial shields.

the inner ring next the dorsocentral are radial in position (fig. II, 2). In these plates we have, I believe, the representatives of the dicyclic base of *Marsupites* and other Crinoids, viz. a proximal ring of under-basals hitherto unknown in any of the Echinozoa, and a distal ring of interradial plates<sup>1</sup> corresponding to

<sup>1</sup> These must not be confounded with the interradial plates seen by Agassiz in *Ophiopholis bellis*, and compared by him to the interradial plates of an Asterid ('Embryology of Echinoderms,' p. 18, fig. 29). The former.



the basals of the Crinoid and the genitals of the Asterid or Urchin, which have not been previously discovered in an Ophiurid. This latter point will naturally be denied by Ludwig, who regards the genitals of an Urchin or Asterid as represented by the orals of an Ophiurid or Crinoid, since they are all perforated by the primary water-pore of the larva. In the larval Asterid the water-pore occupies the same position relatively to the plate which it perforates, as in the Ophiurid and Crinoid.

Fig. III is a projection of the calyx of an *Antedon* larva as seen

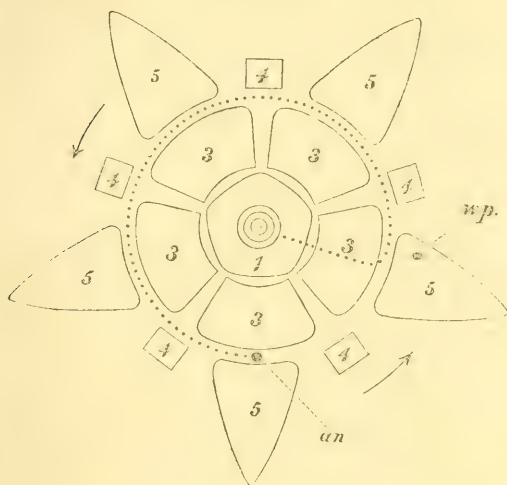


FIG. III.—Apical system of an early Crinoid larva, in which the radials (4) are still very small. The stem-joints are represented as telescoped into one another, so as to bring the terminal plate of the base of the stem or dorsocentral (1) into close relation with the basals (3). The course of the gut from mouth to anus (*an*) is indicated by the dotted line and arrows. *w.p.* Water-pore.

from the dorsal side when placed in the natural position of the situated outside the radials of *Ophiopholis*, are really the orals; while the latter, situated inside the radials of *Asteracanthion*, are the genitals. Following Agassiz, I have wrongly described the former as genitals or basals (this *Journal*, vol. xviii, p. 369), though his figure should have indicated his error to me; and I am sorry to say that this statement was reproduced in the 'Comparative Embryology' of my lamented friend, the late Prof. Balfour (p. 469). As I did not notice the error till after reading over the proof of the chapter on Echinoderms, I therefore take this opportunity of correcting it. I believe, however, that basals are really present in *Amphiura squamata*, though I should say that Ludwig expresses no opinion respecting the homology of the plates discovered by him, and must not therefore be considered as responsible for the views enunciated above.

adult, *i. e.* with the anal interradius backwards, and the course of the gut is shown by dotted lines. Ludwig showed two years ago<sup>1</sup> that the water-pore is in the same interradius as the fore-gut, viz. the right posterior one, or south-east as it may be called. When seen from the dorsal side, the gut winds in a direction opposite to that of the hands of a watch, *i. e.* from right to left, and the water-pore is in the next interradius beyond the anus. The gut of the larval *Asterina* winds in the same direction as that of the Pentacrinoid; and when seen from the dorsal side the madreporite of the Starfish, larval and adult, occupies precisely the same position with respect to the anus as that of the Pentacrinoid does<sup>2</sup> (fig. IV, *w.p.*). Does not this go to

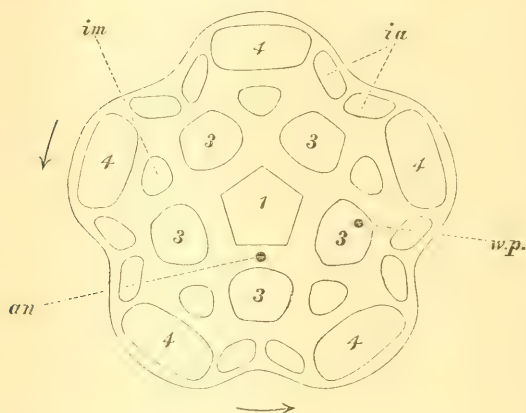


FIG. IV.—Apical system of a larval Asterid (*Asterina gibbosa*), in which the water-pore (*w.p.*) comes into relation with a genital plate (3) at a comparatively early stage of development, slightly altered from Ludwig. *ia*. Interambulacral plates. *im*. Intermediate plates. The course of the gut is indicated by arrows.

prove that the dorsal side of the Starfish corresponds to the dorsal side of the Crinoid, and not to the ventral side, as it must according to Ludwig's theory?

Both in the Crinoid and in the Ophiurid the primary water-pore pierces the oral plate at a point which is not in the middle line of the plate; but, as seen from the dorsal side and counting in a direction opposite to that of the watch-hands, it is slightly

<sup>1</sup> 'Zeitschr. f. wiss. Zool.,' Bd. xxxiv, pp. 315-318.

<sup>2</sup> Ludwig, for embryological reasons, regards the anal interradius of a Starfish as anterior. Abler pens than mine will doubtless discuss this question. I have simply "orientirt" the Starfish in correspondence with the natural position of a Crinoid.

displaced towards the next radius (figs. I, III, *w.p.*). The water-pore of the Asterid larva has precisely the same relation to one of the genital plates (fig. IV, *w.p.*). When it first appears it does not communicate only with the hydrocœl, as it does in the later larvæ and in the adult, but with the left division of the enterocœl, from which the hydrocœl eventually originates. In the Crinoid this condition is the permanent one. The primary water-pore and the numerous pores subsequently developed all open into that part of the body cavity which is developed from the left peritoneal tube. *Rhizocrinus* has but one in each inter-radius; but this is not the case in the allied genus *Bathycrinus*, which has several, and also several water-tubes depending into the enterocœl from the oral ring. The aberrant genus *Hyocrinus* presents some singular variations in this respect.

In both the specimens which I have examined there are two pores in the oral plate of the anal interradius, and there are no others in any of the anambulacral plates which lie between it and the edges of the radials. This is interradius No. 1 of Ludwig's Asterid nomenclature.

In the other interradii the disposition of the water-pores is as follows:

| Interradius. | Oral Plates. |    | Anambulacral Plates. |    |
|--------------|--------------|----|----------------------|----|
|              | A.           | B. | A.                   | B. |
| No. 2 . .    | 0            | ?  | 2                    | 4  |
| „ 3 . .      | 0            | 1  | 3                    | 9  |
| „ 4 . .      | 0            | 1  | 3                    | 7  |
| „ 5 . .      | 1            | 1  | 2                    | 7  |

Thus, therefore, one specimen has all the oral plates perforated by water-pores, with one possible exception; while in the other it is only in the anal interradius and in the next beyond it which contains the fore-gut (No. 5) that the oral plate is pierced by a water-pore. It is significant that this interradius (No. 5) is that in which the primary water-pore appears in larval Asterids, Ophiurids, and Crinoids; and also that in both the specimens of *Hyocrinus* the water-pore is a little beyond the middle line of the large oral plate, just as in the larvæ described by Ludwig. I am bound to admit that these facts may be thought to support Ludwig's theory, but I do not think that they do when considered by the light of its results.

The point on which I lay stress, as Ludwig seems to do also, is the radiate symmetry of the animal. If a Crinoid and Starfish

(both having the anal interradius backwards) be "orientirt," according to Ludwig's theory of their homologies, viz. the former resting on its dorsal and the latter on its ventral face, the position of the water-pore is south-west in the Crinoid or beyond the anus to the watch-hand; but in the Starfish it is south-east or before the anus to the watch-hand (fig. 1v). Turn the Crinoid over, however, so that it has its dorsal surface upwards like the Starfish (fig. 111), and the water-pore is south-east in both cases, *i. e.* in the right posterior interradius.

According to Ludwig, the perforation of one of the skeletal plates by the water-pore is a primary character, and fixes its homologies once and for all.

So far as this relates to the radial symmetry of the Echinoderm I fully agree with him. But may not the precise position of the madreporite depend upon the future relations of the plates of the skeleton, and on the times at which they respectively appear? Thus, in the Asterids the rudimentary genital plates are either relatively large, extending right out from the dorsocentral to the edge of the disc, or they are smaller, as in *Asterina*, and develop near the edge at a little distance from the dorso-central. This is in correspondence with their relations in the adult, while the radials appear beyond them, to be carried outwards on the growing arms. Even in *Asterina*<sup>1</sup> "Der Rückenporus liegt also ursprünglich nicht in der späteren Madreporenplatte, sondern links von derselben;" and Ludwig admits that in other Starfishes there is no primary connection between the water-pore and any genital plate. In the larva figured by Metschnikoff,<sup>2</sup> which has four pairs of spines on each ray, a well-developed madreporic plate is situated at the edge of the disc, quite outside the circle of genital plates.

In the young *Asterias glacialis*, according to Lovén,<sup>3</sup> the water-pore does not appear in its genital plate till after the Starfish is more than 2 mm. in diameter, and there are at least three spiniferous plates between the genitals and radials. In the young *Asteracanthion* of four months, figured by Agassiz,<sup>4</sup> which has three pairs of tube-feet, the madreporic body is still on the edge of the disc of the lower or actinal side; and even in the individual one year old which he figures there is no trace of the madreporite on the dorsal side.

One would greatly like to know the position of the madrepo-

<sup>1</sup> Loc. cit., p. 50, Taf. iii, fig. 41.

<sup>2</sup> 'Studien über die Entwicklung der Echinodermen und Nemertinen,' 'Mém. St. Petersb. Acad.,' vii<sup>e</sup> serie, tome xiv, No. 3, pl. xii, fig. 1.

<sup>3</sup> Loc. cit., p. 87, figs. 257, 259, 260.

<sup>4</sup> 'North American Starfishes,' pl. vi, fig. 10.



rite in these young Starfishes relatively to the rudiment of the so-called "odontophore," which Ludwig considers to be homologous with the orals (mouth shields) of Ophiurids.<sup>1</sup> In *Brisinga* this plate bears the madreporite; and I cannot understand how Ludwig, knowing this fact and regarding the "odontophore" as an oral plate, can have framed his theory of the homology of the orals of Ophiurids with the genitals of Asterids (including *Brisinga*). According to Perrier's observations<sup>2</sup> on the young of this genus there are large interradian pieces separated from the dorsocentral by smaller plates. They are constantly pushed towards the margin of the disc, become reduced in size, and eventually constitute the odontophores, one of them bearing the madreporite. Now Ludwig, for other reasons, considers the odontophores of *Brisinga*, and the Asterids generally, as homologous with the orals of Ophiurids and Crinoids, and their relation to the madreporite supports that view. How, then, can these last be also homologous with the genitals of the Asterids, as his theory asserts? Must not the genitals of *Brisinga* be sought for among the smaller plates between the future odontophores and the dorsocentral, just as in the Ophiurids (figs. 1, 11, 3)? It is hardly likely that the odontophore of an ordinary Asterid is primarily an oral, while that of *Brisinga* is a genital. Does not *Brisinga* represent an intermediate stage between ordinary Asterids with large genitals (basals) and Ophiurids with large orals and small basals?

In the Ophiurid (figs. 1, 11) the proximal ring of interradian plates (basals) is quite insignificant in character, and only develops late, long after the appearance of the radials. These are at first in immediate contact with the dorsocentral, so that there is room between them and the edge of the disc for the orals, which are next the radials in order from the abactinal centre; and as far as can be judged from the figures of Agassiz and Ludwig, they appear to develop before the water-pore which pierces them from the first.

Lastly, in the Crinoid (fig. 111) the basals appear early, and so do the orals which rest upon them, but the radials which ultimately separate the two rings are only developed much later; so that here again the orals are quite close to the abactinal centre, and therefore to the water-pore.

One result of Ludwig's theory is to give the dorsocentral

<sup>1</sup> I use the word "odontophore" for convenience sake, though I fully agree with Ludwig's strictures on the name. But it is at any rate better than "Erstes intermediäre Interambulacralstück." If it represents the mouth-shields of Ophiurids and the orals of Crinoids, why not call it an oral at once?

<sup>2</sup> "Note sur les *Brisinga*," 'Comptes Rendus,' t. xcv, pp. 61-63.

plate and basals of a Crinoid no representatives in the apex of a Starfish or Urchin; while the subanal plate of an Urchin is unrepresented in the Crinoid, and the striking resemblance between the calyx of a Crinoid and the apical system of an Urchin or Asterid goes for nothing. I still believe, however, that the relations of the plates to the enterocœl sacs of the larval Echinoderm are fundamentally more important than the position of the particular plate which comes into ultimate relation with the water-pore. The genitals of an Urchin or Asterid and the basals of a Crinoid are all developed spirally around the right (abactinal) enterocœl; while the orals of a Crinoid or Ophiurid appear in a similar spiral around the left (actinal) enterocœl. Until Ludwig can show good reason for neglecting these facts, and can explain away the various inconsistencies involved by his theory, I shall continue to regard the abactinal system of a Crinoid as homologous with that of Urchins and Starfishes.

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*The VERTEBRATION of the TAIL of APPENDICULARIÆ.*

By E. RAY LANKESTER, M.A., F.R.S.

A PROMINENT objection to the association proposed by me of the Tunicata as a subdivision "Urochorda" with the "Cephalochorda" (Amphioxus) and the "Craniata" in one group, for which the name "Vertebrata" is retained, has been the fact especially put forward by Gegenbaur, that the Urochorda (Tunicata) do not exhibit any "vertebrate" structure, that is to say, a division of the body into myotomes. For a similar reason Balfour, whilst accepting the three-fold division of the Vertebrata, has proposed to denominate that group "Chordata," and to restrict the term Vertebrata to the craniate division of the Chordata, thus excluding the Cephalochorda, as well as the Urochorda from the application of the term "vertebrate."

The best way of indicating the relationship of the Tunicates to the forms usually recognised as "Vertebrata" appears to me to consist in introducing the Tunicata or Urochorda into the long-established and familiarly known group of Vertebrata, in accordance with our improved knowledge as to the actual structure of the former, and *not* in making a new group with a new name, whereby the significance of the association is, to a large extent, lost.

So far as the term "vertebrate" is concerned, we might, of course, legitimately disregard its signification. There is no reason why some Vertebrata should not be inaccurately described as "vertebrate," just as some Arthropoda are not arthropodous, some Chætopoda not chætopodous, and some Gastropoda not gastropodous.

On the other hand, if we accept the term "vertebrate" not as implying the possession of bony or cartilaginous vertebræ (as Balfour proposed), but as meaning "jointed" or "metamerised," then it appears that even amongst the Urochorda or Tunicata, facts justifying its use in relation to them may be discovered.

Apart from the verbal question, it is a matter of some importance that indications of metamerised structure should occur in the Urochorda.

Some years ago (1871—72) I made some observations at Naples on the structure of *Fritillaria furcata*, which satisfied me that the musculature of the tail of that animal is broken up

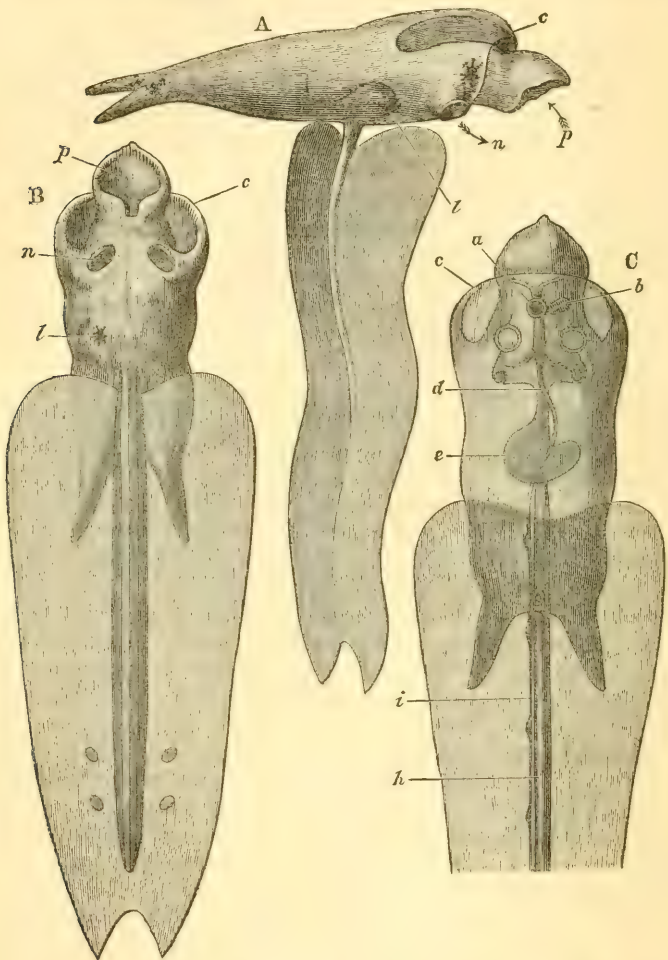


FIG. 1.—*Appendicularia (Fritillaria) furcata*. Surface views. Magnified about seventy diameters. A. Lateral superficial view of *Fritillaria furcata*. B. A similar view from the ventral surface. C. A similar view from the dorsal surface. In this figure the alimentary tract and the nervous axis are seen through the transparent integuments.

into a series of myomeres, seven in number, one corresponding to each pair of nerves given off by the axial nerve-cord. The drawings



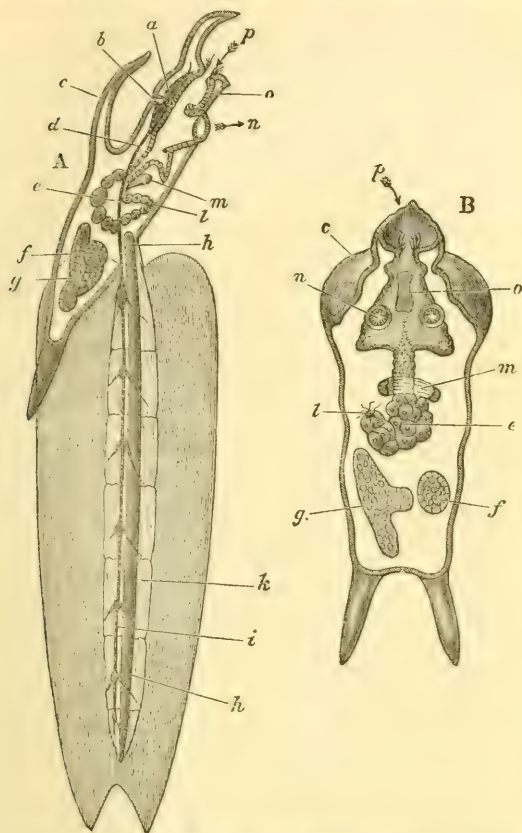


FIG. 2.—*Appendicularia (Fritillaria) furcata*. Diagrammatic views of internal organs. A. Diagrammatic vertical longitudinal section of the "body" of *Fritillaria furcata*. The "tail" is not supposed to be cut, but is drawn as a transparent object. B. A similar drawing of a horizontal longitudinal section, the tail not being represented.

The italic letters have the following significance in all five drawings:—

- a*. The auditory vesicle or otocyst. *b*. The olfactory pit. In contiguity to *a* and *b* is the oblong anterior ganglionic mass of the nervous axis.
- c*. The hood formed by a broad process of the dorsal integument.
- d*. The tubular nervous cord or axis, where it passes from the anterior to the posterior ganglionic mass on the right side of alimentary canal.
- e*. Stomach. *f*. Ovary. *g*. Testis. *h*. Notochord (urochord). *i*. The tubular nervous axis in the caudal region, lying to the left side of the notochord and exhibiting seven slight enlargements, which, excepting the anterior (posterior ganglionic mass), are devoid of nerve-ganglion cells.
- k*. Fifth muscular segment or myomere of the tail. *l*. Anus to the right of the median line, on the ventral surface. *m*. Heart, consisting of two cells only, united by fibres. *n*. Right and left branchial slit (a pair of circular ciliated orifices leading from the pharynx to the external surface). *o*. The endostyle or glandular groove. *p*. The mouth.

which I made at that time have not hitherto been published, though used by me in illustration of lectures. Recently these drawings have been made into woodcuts for illustration of a work on the Lancelet, with which I am busy, and I am indebted to the publishers (Messrs. Kegan Paul and Co.) for the opportunity of submitting these drawings on the present occasion. The fact of a segmentation of the musculature of the tail of Appendiculariæ into myotomes has, I believe, been quite recently recorded by Langerhans, but I am unable to refer more exactly to his observation. A similar segmentation of the musculature seems to occur in some Ascidian tadpoles, though I am not aware that it has been described in detail.

The metameres of the musculature in the tail of *Fritillaria furcata*, shown in the woodcut Fig. 2 A (where *k* points to one of the myomeres), is not obvious in the living or even in a recently killed individual. The division between the separate groups of muscular fibres only became obvious after the specimens had been for some time mounted in glycerine, having been first treated with picric acid as they lay upon the object-slide. I could not trace a distinct fibrous septum separating the myomeres from one another, but merely a break in the continuity of the muscular fibres. It is probable that a very delicate membrane separates each myomere from its successor, but my specimens did not enable me to distinguish such.

As is well known, the nerves which pass off from the nervous axis of the tail do not proceed from ganglionic masses of nerve-cells situated on that structure, but simply from the fibrous nerve-cord, only the first pair having a ganglionic structure at their point of origin. Hence the repetition of paired "spinal" nerves in the tail, though corresponding to the seven myomeres or muscular segments, has less significance as an indication of metamerism than would be the case were there a repetition of nerve-cell-masses in each segment.

The metamerism of the tail of *Fritillaria*, though it may be viewed as an incipient formation of vertebral segments, appears to be most satisfactorily explained as a remnant of a more fully expressed "vertebration," which was possessed by a larger and more elaborate ancestor of the Appendiculariæ, of which existing forms are the reduced and degenerate descendants. The exceedingly small number of cell-units which build up the structure of existing Appendiculariæ, and the whole history of the development of the Ascidians, seems to favour this supposition.

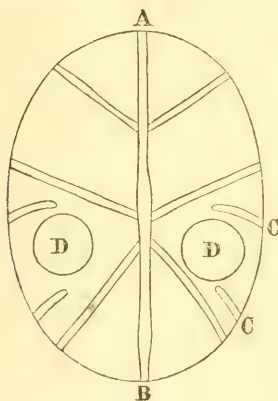
The remaining structures shown in the diagrammatic woodcuts illustrating these remarks are explained in the list of references.

NOTES on the STRUCTURE of SERIATOPORA, POCILLOPORA, CORALLIUM, and TUBIPORA. By Professor H. N. MOSELEY, F.R.S.

*Seriatopora and Pocillopora.*

WHEN Professor Louis Agassiz had discovered that *Millepora* was a Hydroid, and not an Anthozoan, he concluded from the fact that *Pocillopora* and *Seriatopora* were provided like it with tabulæ, that these genera also were to be assigned to the Hydroida. Quoy and Gaimard had, however, figured the twelve tentacles of *Pocillopora*; Verrill examined the coral in 1872, and showed that it was clearly Madreporarian; and I subsequently also examined its structure myself. Although from the close resemblance of the corallum of *Seriatopora* to that of *Pocillopora* it seemed almost certain that the former was also Madreporarian, the fact has never hitherto been proved, nothing as yet, as far as I know, having been published concerning the structure of the soft tissues of this genus. I have, therefore, been on the watch for specimens of *Seriatopora* preserved in spirits, and have just found an excellent specimen of *S. subulata* amongst a most valuable series of objects collected at Zanzibar by Mr. G. Gulliver, jun., M.A., M.B. The coral proves to exhibit peculiar structures of very great interest. It is clearly Madreporarian. The polyps, which are oval in outline, bear twelve short tentacles with rounded knobs disposed in two series. When these tentacles are retracted they are covered over by the indrawn margins of the disc, as in *Actinia*. There are twelve mesenteries only, of unequal depths, two of which bear very long mesenterial filaments, whilst the others appear totally devoid of filaments. The inter-mesenterial chambers are closed by the body-wall beneath, forming a series of pouches, which fit into the conical depressions in the inter-septal spaces of the calices of the corallum. They are of various lengths, to suit the varying depths of these depressions. Two of them are enormously long, and in the decalcified lamina of soft tissue removed from the corallum are seen, when this is viewed from beneath, projecting from the under surface of each polyp like a pair of long conical horns. These are the prolongations of the walls of those inter-mesenterial chambers which

contain the single pair of long mesenterial filaments. These elongate chambers are invariably in the same position in all the polyps, being the right and left median lateral chambers, and the mesenteries bearing the filaments are the ventral ones of each of these pairs. They fit in the recent state into two very deep conical pits formed in the floors of the interseptal spaces between the calcareous septa, which are disposed second and third, right and left, from the median inferior or ventral septum. This pair of conical pits in each calicle is extremely deep, and after the corallum has been treated with acid for some time, and all other traces of the calicles have been dissolved away, the pairs of pits remain conspicuous, still disposed in rows all over its surface. The pits are situate in the inferior or ventral region of each calicle, just inferiorly to its shorter diameter. The calicles are all disposed, as is well known, with their longer axes parallel to the lengths of the branches. Each calicular cavity is divided by a median plate or ridge along its long axis into two halves. Passing to this plate, which has been regarded as an elongate columella by Milne Edwards, from the margins of the calicle are three pairs of septal ridges, disposed right and left, which divide up the two halves of the area of the calicle into an irregular series



Diagram, showing the arrangement of the septa in *Seriatopora* A, superior or dorsal extremity of the calicle; B, inferior or ventral extremity; C C, smaller rudimentary septa; D D, mouths of the pits which contain the pouches with the mesenterial filaments.

of chambers, the arrangement of which in the superior or dorsal region differs very much from that in the inferior. Two further pairs of ridges, which are small and almost rudimentary,



complete the division of the area of the calicle into twelve chambers.

The chambers all have a tendency to have their floors prolonged into conical pits, but it is only in the case of chambers corresponding to the long mesenteries that these are of any great depth. The mesenterial muscles are attached at the bottom of the chamber-pits; those of the two mesenteries bearing filaments are very long, reaching to the very bottoms of the deep pits to be attached there.

To the mesenteries which bear the filaments appear to be confined the production of the generative elements. I have seen no trace of generative cells except on these mesenteries. The sexes appear to be separate in *Seriatopora* stocks, and my specimen is from a male stock. Another remarkable fact in the structure of *Seriatopora* is that the cavities of its polyps are in communication with one another by means of a canal system forming a network. The network traverses the entire area at the surface between the polyps. It forms a close meshwork in the intervening areas, and has in connection with it here and there a few long, nearly straight, larger canals, which run for the distance between three or four polyps to bring the various regions into more direct connection. The meshwork joins the polyp cavities by means of offsets disposed radially to the centres of the polyp areas, two small radial canals usually entering the periphery of each inter-mesenterial chamber, there should be thus twenty-four canal communications with each polyp cavity; but in fact there are usually a few less than this through slight irregularity, about eighteen to twenty-two. The canal system is excavated in the mesoderm and lined by large endoderm cells. It lies all in a single plane parallel with the surface of the corallum. The ramifications of the canal system occupy the grooves on the surface of the corallum between the ridges and small prominences with which it is covered. The radial canals joining the polyp cavities pass between the small projecting points surrounding the margins of the calicles. The only other Zoantharian in which such a vascular network has as yet been described as far as I know is *Stylophora digitata*, in which it has been shown to exist by Koch,<sup>1</sup> who figures it only as seen in vertical section. It appears not to be present in all compound Madreporarians, for Heider<sup>2</sup> has found nothing equivalent to it in *Cladocora*. It is very similar to the well-known canal system in Alcyonarians, excepting that these latter usually ramify in several planes. In *Seria-*

<sup>1</sup> G. v. Koch, "Anatomie von *Stylophora digitata*," 'Jenaische Zeitschrift,' Bd. xi, S. 380.

<sup>2</sup> A. v. Heider, "Die Gattung *Cladocora*," 'Sitzb. der k. Acad. der Wiss.,' 1881, S. 662.

topora it becomes, when spirit specimens are decalcified, beautifully injected with the resulting gas, and all its network is then rendered plainly visible. Exactly the same result occurs when specimens of *Corallium rubrum* are decalcified, the main canal network becoming similarly distended and rendered conspicuous.

On finding the above described structures in Seriatopora, I have again examined some microscopic preparations of Pocillopora. The polyps in Pocillopora appear, like those in Seriatopora, to have only a single pair of very long mesenterial filaments, and these filaments appear to correspond in position to the two long ones in Seriatopora, that is to say, to belong to the central mesenteries of the lateral chambers. I suspected long ago that Pocillopora had only a single pair of mesenterial filaments in each polyp, but I did not feel certain, because these filaments are not enclosed in prolongations of the chamber-walls, as in Seriatopora, and they are so excessively long that in decalcified preparations those belonging to adjacent polyps become interlocked and supposed, so as to render exact recognition difficult, and I did not devote time to the point. As stated, there are no such conical prolongations of the walls of the inter-mesenterial chambers in Pocillopora as exist in Seriatopora, but in the bottom of some of the calices in some Pocilloporas a pair of deepish pits may be made out in corresponding position to the deep pits of Seriatopora, and evidently serving for the reception of the single pair of long filaments. An arrangement of rudimentary septa, closely similar to that in Seriatopora, may also be made out with difficulty in some Pocillopora calices. I have not been able to find generative elements in any specimens of Pocillopora which I have examined.

There is a canal network in Pocillopora like that existing in Seriatopora, but in the species which I have as yet examined it differs from it in that its vessels are wider and coarser, and show a tendency to degenerate into lacunar spaces.

The presence of the deep pits in Seriatopora for the reception of the single pair of generative mesenteries and their hypertrophied mesenteries may possibly explain the pits occurring amongst the septa of some palæozoic corals which may have had a similar function.

The indications so plainly marked in the coralla of both Seriatopora and Pocillopora had already shown that in these compound forms of the Madreporia at least there exists, as in the Aleyonaria, a very definite and uniform orientation of the polyps in each colony, in accordance with the bilateral symmetry and dorsal and ventral differentiation of their structure. This fact, indicated by the arrangement of their septa, is fully borne out by the structure of their soft tissues.

The longer axis of the mouth is always in these corals directed parallel to the direction of extension of the branches, and the polyps are so disposed that the mesenteries bearing filaments are placed always in the direction of the bases of the branches. Similar indications of a regular orientation of the polyps are to be seen in the coralla of the genus *Madrepora* and other genera. Although in *Cerianthus* and *Edwardsia* a dorsal and ventral differentiation of structure is well marked, there is no indication of such in the Actiniadæ, excepting in their early stages of development, and in very many simple and compound *Madreporaria* there seems to be no indication of such in the coralla.

In the Turbinolidæ, for example, there is no trace of such differentiation, and there is also none in the Oculinidæ. It seems probable, therefore, that the affinities of *Seriatopora*, and *Pocillopora* which is certainly nearly allied to it, do not lie with this latter family; but it is almost useless to speculate as to what their real position amongst the *Madreporaria* may be until the structure of the soft parts of more representatives of this group are known. In not having their mesenteries disposed in pairs, with regard to the septa, they differ from other *Madreporaria* as yet investigated.

It is worthy of remark that the pair of mesenterial filaments present in *Seriatopora* and *Pocillopora* cannot, according to the system of development of the six pairs of mesenteries of *Adamsia diaphana* discovered by the brothers Hertwig,<sup>1</sup> correspond with the first pair of filaments developed in the larva according to Lacaze Duthiers, viz. those belonging to the primary pair of mesenteries of the larva.

In *Adamsia*, according to the Hertwigs, the lateral pair of mesenteries are the fifth and sixth pairs in order of appearance, and probably, though they make no statement on this point, the mesenterial filaments belonging to them follow the same order in development. According to Lacaze Duthiers' account of the order of appearance of the mesenteries in Actiniadæ the mesenteries bearing the filaments in *Seriatopora* would correspond with his primary ones if the orientation of most of his figures were transposed, and the position taken up by the primary mesenteries regarded as ventral instead of dorsal when compared with *Seriatopora*.<sup>2</sup> There are at present no reasons for orienting these solitary forms in one way rather than the other, and it will only be possible to obtain such reasons by tracing completely the development of some compound *Madreporarian*, such as *Pocillopora*.

<sup>1</sup> O. and H. Hertwig, "Die Actinien," 'Jenaische Zeitschrift,' 13 Bd., 4 Heft, 1879, S. 545, Taf. xvii, fig. 3.

<sup>2</sup> See Lacaze Duthiers, 'Arch. de Zool. Exp. et Gen.,' vol. i, 1877, p. 59, fig. 6.



It is not at all improbable that the presence of a single pair of mesenterial filaments only in the forms under consideration may have no more ancestral significance than the survival of the dorsal pair only in the siphonozooids of Alcyonarians, and in the young Pocillopora possibly traces of all twelve filaments might exist. An ancestral twelve has probably been reduced by natural selection to the present two, but it would be of interest if it could be shown that the pair thus surviving is that which is earliest developed in the larva.

I hope soon to publish a fuller account of the structure of Seriatopora, accompanied by figures. I have as yet had no time to prepare any sections.

To sum up, Seriatopora is undoubtedly Madreporarian.

The important points in its structure are the presence in it of a highly-developed vascular system similar to that existing in Alcyonarians; the fact that each polyp has only a single pair of mesenterial filaments, and that these filaments are lodged in pouches resting in special deep pits in the corallum, that only the mesenteries bearing the filaments develop sexual organs, that the coral stocks are unisexual, that in the structure of the polyps a differentiation into a superior or dorsal and inferior or ventral region is clearly indicated both in the soft tissues and in the corallum, and that the polyps in each stock are arranged with regularity with the dorsal ends of their oval calicles turned towards the tips of the branches, and their longer axes parallel to the lengths of the branches, in this respect agreeing with Alcyonarians.

#### *Corallium rubrum.*

When examining the soft structures of *Corallium rubrum* by means of decalcification, in the beginning of the present year, I paid special attention to the small polyps which appear as minute white points scattered on the cœnenchym around and amongst the main large sexual polyps. I found that they are small polyps devoid of tentacles, just as described and figured by Prof. Lacaze Duthiers in his classical memoir, but I further noticed facts which he does not mention, namely, that these polyps have their dorsal and ventral pairs of mesenteries deeper than the lateral pairs, and possess only a single pair of mesenterial filaments, the dorsal. Hence they agree in every particular in structure with the siphonozooids<sup>1</sup> of Sarcophyton and other Alcyonarians, in which two kinds of polyps, autozooids and siphonozooids, occur. Prof. Lacaze Duthiers paid especial atten-

<sup>1</sup> "Report on the Scientific Results of the Voyage of H.M.S. Challenger," 'Zoology,' vol. ii, 1881, p. 118.



tion to these zooids, as they had apparently been supposed before he made his distinguished investigations to be water-pores in communication with the canal system. He shows that they are not of that nature, but describes them as buds from which the large sexual polyps are developed, and he actually watched the process of the growth of the one form into the other in specimens which he kept living in glass vessels. Singularly enough, however, I have been unable to find intermediate forms on any of the specimens I have examined between these minute polyps and the larger forms. I find none of the small ones with traces of tentacles, and as far as the very small quantity of material I have had at my disposal goes, I have not been able to verify Prof. Lacaze Duthiers' statement that the minute polyps are most abundant towards the growing tips of the branches of the coral.

I have failed to obtain a further supply of *Corallium* from Naples this year, and therefore I publish this note in order that attention may be drawn to the matter. Kölliker has described in *Haliseptum* and *Virgularia* the existence of certain undeveloped zooids which, occupying the lower and younger parts of the stocks, produce the generative elements, and apparently as growth proceeds become developed into fully-formed autozooids, relinquishing at the same time their generative function. These zooids have traces of the two dorsal mesenterial filaments only, and otherwise, excepting in the possession of generative organs, correspond to ordinary siphonozooids. The mode in which they attain the remaining six pairs of filaments after ceasing to perform the generative function is not known. The undeveloped zooids in *Corallium rubrum* are so abundant, and there are so many of them constantly present, all in the same siphonozooid stage, that we should apparently be justified in regarding this Alcyonarian as polymorphic, like so many others composed of two kinds of zooids, with this peculiarity, that any of the siphonozooids are capable, like the sexual zooids of *Haliseptum*, of being developed into autozooids.

A new form of *Corallium* (*C. stylasteroides*), lately described by Mr. Ridley<sup>1</sup> of the British Museum, has much the appearance of having distinct minute siphonozooids disposed regularly between the autozooids. Mr. Ridley agrees with me in considering them as such. The occurrence of the two kinds of polyps amongst Alcyonarians is much more common than was once supposed, and is by no means confined to the Pennatulids.

<sup>1</sup> "On the Arrangement of the Coralliidae, with Descriptions of New or Rare Species," 'Proc. Zool. Soc.,' 1882, p. 225, Pl. IX, fig. 2.

*Tubipora.*

On examining the infundibuliform tabulæ of *Tubipora*, to which attention has been drawn by Mr. Charles Stewart,<sup>1</sup> and by myself,<sup>2</sup> I find that the occurrence of these axial internal tubules is very irregular. In some species of *Tubipora* they seem almost entirely absent, and apparently their abundance varies in different specimens of the same species. In some few cases I have detected one infundibuliform tube lodged within another, so that the structure is exactly similar to that occurring in *Syringopora*. In some cases the tubes at their lower extremities give out a series of radial offsets which have somewhat the appearance of septa when viewed from below.

My assistant, Mr. Sydney Hickson, is engaged in preparing a memoir on the structure of *Tubipora* and other Alcyonarians. In this the form and relations of the curious infundibular tubes of *Tubipora* will be thoroughly worked out, and some new instances of polymorphism amongst Alcyonarians will be described.

<sup>1</sup> C. Stewart, "On a New Species of *Stylaster*, with a Note on *Tubipora*," 'Journal of the Micro. Soc.,' 1879.

<sup>2</sup> "Report on Scientific Results of the Voyage of the 'Challenger,'" 'Zoology,' vol. ii; 'Report on Corals,' p. 125.

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NOTE on PACINIAN CORPUSCLES. By VINCENT HARRIS, M.D.  
 Lond.; Demonstrator of Practical Physiology at St. Bartho-  
 lomew's Hospital. With Plate XXXII.

IN one of last year's journals<sup>1</sup> I called attention to the presence of the corpuscles of Pacini or Vater in great numbers in the pancreas of the cat and in the lymphatic mesenteric glands of the same animal. To that note I desire to add the following facts:

*Thyroid gland of the kitten.*—My attention was called by Mr. Pettifer to some sections of the thyroid gland of a kitten, which contained several well-marked examples of the corpuscle in question. They appeared to me, although situated in the very midst of the gland, to be connected with the connective-tissue framework rather than with the gland tissue proper. Their structure was very distinctly seen, and their size was large. I am unaware of any previous notice of Pacinian corpuscles having been found in this locality.

*Pancreas of dog.*—In my former note I supposed that the presence of Pacinian corpuscles in the pancreas of the cat was to be explained by the fact that in that animal they were especially numerous in the mesentery. I have, however, found in the pancreas of a dog hardened in absolute alcohol, well-marked examples of these corpuscles. They were situated in the very midst of the gland, and not markedly, as in the cat's pancreas, surrounded with connective tissue. Kölliker mentions<sup>2</sup> that these bodies are found in man, invariably on the great sympathetic plexus, in front of and close to the abdominal aorta, behind the peritoneum, *particularly near the pancreas*, frequently also in the mesentery *close* to the intestine; but this observer does not add any further mention of the existence of these corpuscles in the pancreas itself. I append a drawing (Fig. 1) of a corpuscle, chiefly interesting from the fact that it was not far from a mass of ganglion cells (fig. 2), of which I have also added a rough sketch.

<sup>1</sup> 'Quart. Journ. Microscopic. Sci.,' vol. xxi, July, 1881.

<sup>2</sup> 'Manual of Human Histology,' 1852 (Syden. Soc. Trans.), vol i, p. 469.

*Pad of kitten's paw.*—It is well known that Pacinian corpuscles are numerous in the pads of the cat's feet. I would, however, add several observations which I have made on the subject lately. The central pad of the kitten's fore-paw consists of three elevations, of which the central one is the largest, and is separated from the lateral elevations by a slight depression on either side. On section, it is found that there is a distinct fine fibrous septum continued downwards from each depression, and it is low down beneath the subcutaneous fat, on the outside of the fibrous septa, that the greater number of Pacinian corpuscles are found, according to my experience. It is a striking fact that the corpuscles often occur in considerable masses, connected together with fine fibrous tissue. In one specimen cut horizontally I counted no less than sixteen on the outside of one septum and ten on the outside of the other, and I have made a sketch of one section (Fig. 3), which shows no less than sixteen corpuscles. They are much less numerous in the pads in front of and surrounding the central one, and also more numerous in the fore than in the hind paws. It is generally agreed that these corpuscles have nothing to do with the sensation of touch. This, their irregular distribution and aggregation in masses, low down beneath the subcutaneous fat, would certainly prove; but the question as to what their function or functions, if they have more than one, may be is still unanswered.

The only tissue which appears to be almost invariably connected with these bodies, wherever they are found, is the vascular, and it therefore seems possible that they may have something to do with the regulation of the blood supply.

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## REVIEWS.

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“*Ueber den Bau und das Wachsthum der Zellhäute.*”

VON DR. E. STRASBURGER, Prof. an der Univ. Bonn.  
Jena, 1882. A Review by H. MARSHALL WARD, B.A.,  
Fellow of Owens College, Victoria University.

A FURTHER communication on the subject of the vegetable cell, by the author of the well-known book, ‘Zellbildung und Zelltheilung,’ cannot fail to interest all who are busied with morphological investigations. In the present work, from the active pen of Professor Strasburger, we are introduced to a large number of new facts, supplemented by a brilliant series of hypothetical considerations. However opinions may differ as to the value of the latter, there can be little doubt about the importance of the former, and no better plea for special work in biology could be demanded than such a book and its natural connection with previous investigations by the same author.

One of the difficulties met with in this as in former books by Professor Strasburger is the want of obvious arrangement of the multitudinous details. It requires no little patience and some skill to pick out the most important points from a huge mass of minute details spread over more than 250 pages of text, and even the aid of a fairly complete index and tables of explanation to the eight large plates in no way obviates the necessity of laborious reading of the whole work.

This is, perhaps, in itself no fault; but it would conduce to greater clearness on the part of the reader if the author either summed up his results more distinctly, or, at any rate, offered more apparent clues to the directions in which the facts are leading him.

In what may be termed the first section of the book we are introduced to a mass of observations going to prove that the cell-wall increases in thickness by the successive deposition of new layers of ready-formed matter. One is impressed

with the strength of Strasburger's position by the first example selected—*Caulerpa*—which happens to be a singularly fortunate one in many respects, since some of the best observers have investigated and reported upon the structure of its cell-wall. Schacht gave it as affording good examples of growth by apposition. Naegeli, in his large work on starch, stated that an explanation of the course of the layers on the cell-wall and the supporting rods or cords which traverse the cell cavity is not in accordance with the apposition theory. In 'Das Mikroskop' are figures showing (1) how the layers run on the wall and the abutting rod, and (2) how they should run supposing their apposition as layers. Similar views were promulgated by Hofmeister; but Dippel described the layers as arranged otherwise. In 1880 Schmitz pointed out that Naegeli's description is wrong. The course of the layers is exactly the reverse of that given in the above-named figures.

Strasburger's description agrees with that of Dippel, and with the latter—Schacht and Schmitz—he regards the course of the layers as explicable only by apposition. He points out that the actual state of things is that figured by Naegeli as what *should* be, supposing the thickening took place by apposition, thus claiming the support of Naegeli himself.

It appears that the supporting cords which run across the cell cavity, and are perpendicularly attached to the side walls, consist of concentric layers corresponding with those on the walls; that the outer layers of the cord are younger, and run into the innermost and youngest layers of the wall, while the axis of the cord is continued through to the older superficial layers.

Cords running parallel to the length of the cell often become buried in the thickness of the cell-wall, the layers which are deposited over them becoming bulged towards the lumen of the cell as they pass over; the layers outside the cord remain flat.

Explanation of the so-called alternating watery and less watery layers is based on the action of contact surfaces towards the light. Layers may remain separate or become compressed in different degrees. As useful terms, to be consistently employed afterwards, Strasburger gives the following:—*Lamellæ* are the primary structures proceeding directly from the protoplasm; a *layer* is a complex resulting from several superposed lamellæ; the *limiting membrane* designates the altered inner edge of a layer. This alteration may affect the density of one or more layers, and the thickness of the limiting membrane become considerable.

By behaviour on swelling, optical characters, and action towards reagents, the cell-wall betrays the presence of layers, variously altered by compression, &c.; the gradual entrapping and burying of substances in the successive layers demonstrates the apposition of the latter. After referring to further details Strasburger proceeds to describe the origin of the young supporting cords.

In the apex of the "Rhizome" of *Caulerpa* is much dense protoplasm, in which starch and fine granules are distributed; certain granules distributed over the surface of the cell-wall, and in strings of protoplasm proceeding from this, are to be distinguished as giving proteid reactions, &c.; these "microsomes," arranged on the very fine strings which give rise to the supporting cords, remind one of the optical section of a cell-plate just before the membrane is formed.

As will be seen later, Strasburger considers the "microsomes" as elements derived from the protoplasm to form the cell-wall. In the above case they are to be regarded as brought by streams in the protoplasm to the inner surface of the cell-wall, and to the outside of the young cord; here they become arranged into series, and plastered on—so to speak—as "lamellæ."

In young growing internodes of *Clematis* the pith cells are bounded by simple thin walls, which give a blue reaction in zinc-chloride and iodine; only at the intercellular spaces do they appear separable into two layers. In the next lower internode this primary cell-wall has a thickening layer deposited over its inner surface, and separated by a very delicate line from the former; this thickening layer gives the reaction of pure cellulose, but the primary wall has already become cuticularised, and resists acid.

The succeeding internodes have the pith cells thickened by further deposits of similar layers as Dippel described. When older, the outer layers become slightly changed; but faint "separation surfaces" mark them. These separation surfaces are of the nature of "limiting membranes," and appear to be due to the continued action of the surrounding medium during pauses in the apposition of the layers which they separate. In the layers themselves can be seen indications of lamellæ. Careful treatment with  $H_2SO_4$  shows that the cellulose layers dissolve more rapidly than the slightly altered limiting membranes, and at length a network of middle lamellæ—the altered original walls—alone remain. The walls of the older pith cells of *Clematis* are pitted, and Strasburger shows that the pit arises by arrest

of the apposition of layers at these spots. The bottom of the pit is bounded by the original cell-wall, over which a thin layer is continued; the sides of the pit are covered by a limiting membrane, which, however, is no continuation of those between the layers of the wall, but is due to an alteration at the *edges* of these layers as they suddenly stop at the pit.

We have here, therefore, an example of growth by apposition in its simplest form; the layers—each of which must be regarded as formed of several lamellæ—marking periods of active growth, the separating surfaces indicating pauses in the process.

In the young embryo sac of *Ornithogalum*, the secondary thickening layers arise when the sac is full of tissue, proceeding centripetally from the outer cells; all stages can therefore be obtained in one section.

The primary walls of unthickened cells are seen to be clothed by a very delicate layer of protoplasm, bearing "microsomes" which are coloured brown by iodine; at this period the starch granules begin to disappear, and by the end of the thickening process none remains. It is possible to cause the protoplasm to withdraw, leaving the "microsomes" arranged in series on the cell-wall. Such a sheet of microsomes, embedded in protoplasm, is to be regarded as an incipient thickening lamella. The numerous other details agree essentially with what has been stated, and we may pass shortly over the cases of the pith of *Taxodium*, the endosperms of *Phoenix* and *Strychnos*, the hardened endocarps of many stone fruits, &c. Many important observations are to be found in this section, as well as in that embracing the investigations of wood- and bast-cells; and Strasburger finds more and more support for his views here, adducing at the same time difficulties which the theory of intussusception does not at any rate obviously explain.

Among others the very fine canals or pores passing completely through the walls from cell to cell of the endosperm of *Strychnos* should be especially noted; similar continuous canals occur in other cases, and Strasburger seems inclined to lay some stress on these as of more general occurrence. The remarks on *Glaucocapsa* are also important. Space does not admit of our dwelling further on this portion of the book, however, and one more example must suffice as indicating the mode of treatment here pursued.

Professor Strasburger has made an excellent study of the wood-cells in *Pinus*, and since these have also been investi-



gated by Russow, Sanio, and others, the details of a concrete and critical example are offered to us.

Shortly put, the radial cell-walls of the cambium are thicker than the tangential ones, and become relatively thinner (by stretching and loss of water?) as they pass out of the younger zones. In the young wood we find the faintly-continued origin of the well-known "bordered pits" (Tüpfel) on the radial walls only, and in a primitive condition. On the thin plate in the centre arises, as a circular cushion, the "torus;" this is a thickening of the primary wall at this place.

The cell protoplasm now begins to bring "microsomes" to the surface, and the torus becomes thickened. Soon after the ring is formed, which, by increase in breadth, arises obliquely from the edges of the "*primordial-tüpfel*," and changes it into the finished *Hoftüpfel*—the finished "bordered pit."

This ring does not become formed on all the primitive pits, however; many of them disappear later. In other cases a faint circular line around two complete "bordered pits" indicates the remains of a primitive pit on which two rings have formed.

The thickening of the wall of such wood-cells begins with the origin of the rings above described, and proceeds with the growth of the latter; at the same time the primary cell-wall, on which the thickening layers are deposited, becomes chemically changed. The ring consists of continuations of the thickening lamellæ, each passing beyond the edge of the former one; and the thin, altered membrane passing over all and lining the inside of the pit, must be looked upon as arising by alteration of the edges and surfaces of lamellæ exposed to the continued action of their environment.

Space does not admit of our discussing other observations on this difficult histological subject; enough that Strasburger gives a fair *resumé* of earlier and other views. The secondary thickening layers, described above as deposited successively on the primary wall, often exhibit spiral striations; these may be broad or narrow, and commonly cross the long axis at about 45°. Strasburger fails to confirm Naegeli's observation that these spirals may cross one another in the same layer; they do not appear on the ring of the bordered pit, but they may influence the direction of the latter. The striæ may be so close that they can only be seen in  $\text{H}_2\text{SO}_4$ , &c. We must pass over the discussion relating to the earlier views as to the nature of these striæ.

tions and markings. Strasburger finds that the thin layer of protoplasm lining the cell-wall, and applied to its inner layers, carries numerous "microsomes," which become used up as growth proceeds; that these "microsomes" are arranged in corresponding directions with the spirals, &c., and that in alcohol the contracted protoplasm may break up into spiral bands corresponding to series of the granules. He also finds that according to the stage of apposition the "microsome" bearing sheet can or cannot be withdrawn from the cell-wall. At last alcohol does not cause withdrawal of the protoplasm, and no more "microsomes" can be seen; they have all been deposited in successive "lamellæ" to form the thickening layers.

The persistence of the nucleus in these wood-cells till the last may be noted. Many details as to the micro-chemical reactions, &c., must be here passed over. It is important that in the cross-section only concentric lines (the layers and lamellæ) are detected when no spiral striæ occur in the longitudinal surface view. When striæ occur the concentric lamellæ in the cross-section are traversed by radial striæ, giving the appearance as if the lamellæ were made up of rodlets. Where coarse and fine striations coexist in the longitudinal sections the cross-section shows corresponding broad and narrow markings. All these indications of structure pass into one another by every gradation, and are in accord with corresponding arrangements of the "microsomes," whence must follow that they depend upon the mode of building up of the "lamellæ" out of the sheets of "microsomes" brought by the protoplasm.

Reference only can here be made regarding the further observations on the striation and lamellation of ordinary cell-walls. Some important observations on *Spirogyra* and *Cladophora* follow the examination of schlerenchyma fibres, &c., while the epidermis cells and vessels of many different examples afford still further support to Professor Strasburger's view. If the description and figure relating to the perfect agreement of the striæ and "microsomes" on the vessels of *Impatiens* are correct a better demonstration could scarcely be desired.

In that portion of the book dealing with the development of the pollen grains of the Angiosperms and Gymnosperms we need only select one example to illustrate the character and importance of the facts adduced.

In the *Malvaceæ* Strasburger finds that the pollen mother-cells, after dividing into groups of four, begin to disappear; their walls and those of the tetrads become swollen and

colourless, and the protoplasm of each young pollen-cell becomes aggregated on one side, and is connected by strings of protoplasm with the cell-wall.

The "Tapeten-zellen"—inner cells lining the young anther cavity—now swell, pressing the next layers of cells, and starch is found in the epidermal and outer layers of the anther cavity.

The walls of the tetrads then dissolve, and the young pollen grains float free in the anther cavity. Fine granules between the free pollen cells are derived from the late swollen and dissolved cell-walls. These are coloured yellow by iodine and Schultze's solution.

The walls of the pollen-grains become firm and double contoured and are marked by radial striæ, and the nucleus becomes flattened. Then an increase of the protoplasm takes place, and the wall of the pollen grain is found to become rapidly developed in two directions: a *centripetal* growth in thickness is accompanied by a *centrifugal* development of spikes on the outer surface. The thickening layers are deposited on the aforesaid double-contoured and firm cell-wall, and are less refractive and more capable of swelling than it; the spikes arise as small knobs on the outer layer. The separate pollen grains now lie in a matrix, in which protoplasmic strings run from one grain to another, and these strings carry numerous "microsomes." The material for the latter appears to be derived from the late tetrad walls and the now diffusent tapete cells.

These "microsomes" nourish the external parts of the developing pollen grain, becoming aggregated at the surface, and especially where the spikes are forming. The inner, swollen thickening layers are soon seen to be traversed by pores which abut on the outer layer between the spikes, while the superficial lamellæ of the outer layer become differentiated as a special layer, continued over the rodlets which cause the radial striation.

As the pollen-grains approach maturity their nuclei divide, and each obtains two. All the matter derived from the "tapete cells," &c., passes between the young grains, and even the fragments of the broken-up nuclei of the former become used for the nutrition of the latter. As this occurs the protoplasm of each pollen cell rapidly increases in amount, and the vacuoles disappear.

So far, complicated as is its structure, the pollen grain is enveloped by the "extine" only; the "intine" now forms as an independent layer around the protoplasm, and becomes swollen and protruded inwards at places.



About this period peculiar thickenings are formed on the walls of the cells composing the epidermis of the anther, all the food plasma has been used by the pollen, and the grains become "ripe." Sections show that the two nuclei found in a younger stage gradually disappear and become distributed in the protoplasmic network. Strasburger believes this to be important, since it explains why he and Elfving failed to trace the nuclei into the apex of the pollen tube in Angiosperms.

In *Malva* the pollen grain may protrude many tubes; but Strasburger could not find that the nuclei suffer corresponding division. The facts appear to be simply that the nuclear substance becomes distributed in the protoplasm, and that, after passing over to the egg-cell, a reaggregation occurs and a "nucleus"—the nucleus of the male element—is found once more.

The above remarkable results are further supported by investigations of the pollen in *Geraniaceæ*, *Gaurea*, *Clarkia*, *Epilobium*, *Cucurbitaceæ*, and many others, including several *Monocotyledons*. Especially interesting results were afforded by *Cucurbita*.

From many points of view Strasburger's investigation of the spores of vascular Cryptogams—*Lycopodium*, *Osmunda*, *Equisetum*, *Marsilea*, and *Salvinia*, are of great importance. We must, however, refer the reader to the book itself for details, as also for the description of some points in connection with the lower Cryptogams, and pass on, meanwhile, to notice the result of the author's investigations of the starch grain.

What may be termed the second division of the book is presented under the following heading:—"Anlage und Wachstum der Stärkekörner." We may conveniently, as before, select one prominent example as illustrating the chief results obtained.

The large well-marked starch grains of *Phajus* have been well studied by Schimper, and Strasburger's description agrees essentially with his. Strasburger has also come to the conclusion that the dark and bright layers, which vary in breadth, &c., are layers of apposition—that the dark lines denote surfaces of adhesion, and are usually formed between layers composed of many lamellæ. Where a long pause has ensued between the apposition of two layers, the continued action of the environment has altered the outer lamellæ of the older layer; this becomes evident by changes in optical properties, &c. Similarly, the outermost layer of all, which is also the most resistant, has been formed by



alteration due to the environment, and it is important to notice that this layer—of the nature of a “limiting membrane”—is in part formed where the edges of incomplete starch layers overlap.

Strasburger declares that the most resistant part of the starch grain is that which has been longest subjected to the action of the environment; the inner parts vary according to circumstances. By the careful use of reagents, observations on the process of swelling, &c., it is possible to demonstrate radial structure in the separate layers, and a correspondence between the tangential and radial structure is traced similar to what was obtained for the wood-cells in *Pinus*, &c. Naegeli's explanation of the markings and structure of the starch grain is found to be insufficient, and after examining the facts derived from observations on other starch grains—especially those of *Cycas*, *Phaseolus*, Potato, &c.—Strasburger proceeds to another explanation of these and other similar phenomena. Some ten pages are occupied with his views “*Ueber den Bau der Stärkekörner und Zellhäute, und das Verhältniss der Quellungsrichtungen zu dem anatomischen Bau.*”

Strasburger agrees with Schleiden, Unger, Crüger, Schacht, and Schimper, &c., that the starch grain grows by apposition of new layers. Schimper had already shown that new layers can be formed over the nucleus of a corroded grain; but explained them otherwise than by simple apposition, attempting, in fact, to reconcile Naegeli's views as to the alternation of watery and less watery strata with his own views of deposition. The ingenious explanation of the layers in the starch grain offered lately by Arthur Meyer, does not appear to us fully dealt with by Strasburger. The views held by the latter may be fairly summarised as follows.

The central “nucleus” of the starch grain is soft. In general any layer after being covered by another becomes less refractive, depending on its assumption of water; in this manner is explained the fact that the layers become softer and less refractive as they approach the centre of the grain. Those layers which do not become covered by others preserve their density, or become denser; in this way the darker lines become formed during pauses in the growth of the grain.

Since each new lamella is closely and tightly affixed on those beneath, a strain is produced on the former as the latter becomes more watery, and the tendency of the latter to absorb water is favoured by the strains in the starch

grain, which also tends to become enlarged. This addition of water, however, occurs more easily in a tangential direction, and each layer is more or less prevented from enlarging by the other closely adhering layers; thus, as Naegeli found, each lamella suffers a *positive* tension with respect to the next inner one, and is in *negative* tension towards the outer ones. Hence also follows that the cut surface of a halved grain becomes concave, and that the nucleus may become hollow from the tugging exerted by the layers as they increase their surface. The assumption of water can only be great, however, in those layers which pass completely round the grain; hence, in *Phajus* starch those layers which only go partly around the whole grain show no regular increase of watery contents as we pass from the younger to the older parts.

In certain cases the simultaneous action of several causes produce complications into which we cannot here enter. The radial slits, &c., so often found in the completely formed granule, are co-ordinated with the differences in cohesion in different directions. On drying, for example, most water is abstracted from the internal parts, and the cohesion is weakest in the tangential direction; hence we find the resulting fissures are radially arranged, and are wider towards the centre of the grain. So, too, the adhesion of the lamellæ being very strong, pressure and artificial treatment causes no separation of the layers, only the formation of radial fissures. The close correspondence between the structure of a starch grain and of a thickened cell-wall, induces Strasburger to believe that the lamellæ in both cases must result from the union of "microsome" series and sheets; that the cohesion between the rows of "microsomes" of any one sheet is weaker than that between the sheets or lamellæ. In *Pinus* wood-cells, for example, the union between two series of "microsome" rows may be weaker or stronger—as was seen in cases where the sheets broke up into spirals—and so on.

In transferring the hypothesis from the cell-wall to the starch granules, and *vice versâ*, it must of course be remembered that in the one case the lamellæ are plastered on, so to speak, from the inside, while in the other they succeed one another from without. In an ordinary cell the straining from within is balanced more or less by the pressure and elasticity of the external layers; pauses in growth enable the environment to produce changes resulting in the formation of *Grenzhäutchen*, and this is most evident at the external surface, where a cuticularised layer is formed outside,

or a "limiting membrane" at the end of the growing period.

The layers of the cell membrane tend to become extended on the inner face of the wall, those of starch on the outer. Hence, in the membrane, each layer is in a state of *positive* tension towards the next outer, and *negative* towards the inner one. The destruction of membranes also teaches the same lesson as in the case of starch, only we have to deal in many cases with more complex layers, some of which are altered in various ways. For further facts and discussion we must refer the reader to the book itself, and pass on to say a few words concerning Strasburger's treatment of some further portions of the subject.

"*Die Proteinkrystalle*" and "*Scheidewand-bildung*" form the headings to the next two sections.

Protein crystals or "crystalloids" differ from ordinary crystals in being capable of swelling, apparently according to laws similar to those which govern the changes produced by warming many ordinary crystals. Their form is the same from the commencement; and since many have been produced artificially, they can no longer be regarded as growing by "intussusception." Strasburger regards them as differing in no important respect in their origin from ordinary crystals.

In the formation of division walls or laminæ Strasburger considers that the elements of the cell-plate ("microsomes") are not composed of carbohydrates, but approach proteids in their composition, and we are to conclude that they, together with the plasma sheet in which they are embedded, form the septum, much as the thickening layers are formed on a cell-wall. These "microsomes" are carried in the "*Verbindungs fäden*" (and not on their surface), and form the dots so often described in the figures of the process of cell division. We are therefore to view the streams of protoplasm as carrying these "microsomes" to the equatorial region of the dividing mass, where they become mobilised and arranged into a sheet, which forms later the thin primary division-wall.

In some cases, as *Spirogyra*, *Cladophora*, the mobilisation of the "microsomes" is effected without *Verbindungs fäden*; fine streams of "microsomes" move towards the edges of the equatorial ring, and become gradually distributed inwards until a sheet is formed, the "microsomes" and material between them becoming coherent laterally.

We are therefore to accept the consequences that the



cellulose results from the breaking down of protoplasm as a split product, the elements not necessarily being identical in all cases, however, as seems indicated by the differences in character of the membranes produced. Whatever opinions may be held as to the value of these considerations, it is clear that Professor Strasburger has made an excellent attempt to bring a large series of hitherto independent facts into relation with one another and with new discoveries; that the exact nature of the "microsomes" themselves is in the highest degree problematical, it is not necessary to remark. Minute granules in a protoplasmic matrix is always a vague phrase, and we have seen how much Strasburger's interpretation of the nature of the "microsomes" has changed; nevertheless, here are other remarkable relations of time and position, and the large number of facts explained is here, as elsewhere, to be kept in mind as a test of the worth of the hypothesis. Further investigations may be anxiously watched in these directions.

In the section treating of "*das Flächenwachsthum der Zellhäute und die Faltenbildung*," we meet with further evidence of Professor Strasburger's indefatigable powers of observing and thinking, two operations not always found together. This part of the book is for us of especial interest. It is well known that the growth in surface, &c., of membranes has always offered peculiar difficulties, which were considered hardly explicable by the "apposition" hypothesis, and therefore of especial weight as evidence for the theory of growth by "intussusception." Strasburger sets out by stating that the results concerning the thickening processes render it in the highest degree improbable that a different mode of growth is followed here, claiming an *à priori* argument for apposition; and, indeed, all must allow that if the microsome-bearing protoplasm sheets pass directly into lamellæ, it is not easy to suppose that soluble carbohydrates, &c., pass into a layer and crystallise in order to allow of its increase in surface.

Schmitz first pointed out the possibility that by the stretching and pressing together of layers by the growth of daughter-cells, the walls of mother-cells may become extended.

In *Glæocapsa* this occurs until the outer layers are thrown off; in *Cladophora* the pressure and extension produce a homogeneous sheath. In such cases the continual stretching is accompanied by apposition of new strengthening lamellæ, and it becomes a question of how far the outer layers can endure the process; very often they become



changed to cuticles, slimy sheaths, &c.; possibly the alteration in structure is connected with the tensions they have to undergo, as well as the action of the environment.

The tearing away of outer layers in *Microspora*, and the formation of intercellular substance in some Algæ, as well as of caps, &c., support these views.

Schmitz leaves undecided whether intussusception does or does not aid in these processes. Strasburger does not admit this, and considers it only possible, not probable, that protoplasm, penetrating between the cellulose particles (which have arisen as disintegration products of protoplasm), there deposits new microsomes where required.

The professor then proceeds to some observations of considerable interest. In the bark of some Conifers the sieve-tubes are pulled tangentially by the enlargement of the cambium, and are pressed by the cortex and cork until the lumen is obliterated, and a horny, single, inseparable layer represents the tube; in the wood the young radial walls of the cambial cells become thinner as they are stretched, and, although several lamellæ appeared to have been deposited, at length form a single, thin, homogeneous membrane. In other words, by pressure and tension several lamellæ may become fused into a single homogeneous layer.

As in such cases as the passage of zoospores through cell-membranes, the in-boring of parasitic filaments, &c., it may be supposed that protoplasm can soften already dense cell-walls; and Strasburger believes that some such process occurs in the branching of *Cladophora*, where the wall bulges at a certain point, and the before lamellated membrane becomes stretched to a thin, homogeneous one. In unicellular plants, where hydrostatic pressure alone cannot cause curvatures, the curving produced by light or gravitation may be accompanied by softening and stretching of the membrane at certain spots. Weisner says that in positively heliotropic plants the elasticity of the illuminated side is increased, so that the stretching on the shaded side is greater.

Other examples are quoted, as *Ulothrix*, and then some observations on *Spirogyra*. The cell-wall here only presents two layers, an outer one which may become slimy, and an inner, more refractive one, of the nature of a "limiting membrane;" a dark line separates the two. It is interesting to note that in *S. orthospira* the separation of the cells takes place so suddenly and sharply that they may fly apart to some distance.

The free end of such a separated cell soon shows the in-

fluence of the surrounding medium, and grows rapidly, and the colourless protoplasm within shows streams laden with "microsomes" playing exactly as at the incipient cross-septum during the division of the cells, and hurrying towards the terminal inner surface, there to become arranged into a sheet which forms a new lamella. This peculiar, microsome-laden sheet was earlier described by Strasburger as a condition of the outer portions of the protoplasmic body (Hautschicht), in which rod-like elements appear. Fine, hitherto not understood protuberances now seen inside the cell-wall, are explained as already consolidated portions of the layer. As the thickening thus occurs, the caps formed over the growing end are stretched by pressure, and the lamellæ become closely and tightly fused together.

A short section on the formation of membranes in the animal kingdom follows, and need not here be further noticed; it is of interest, however, to observe how comparisons between the two kingdoms can be instituted in this connection.

From this point onwards, the last one fifth of the book is occupied with almost purely hypothetical considerations, and is in most respects decidedly less valuable to the morphological student; nevertheless, the crystallisation of the numerous ideas around the nuclei of facts obtained gives us a clear insight into the peculiar sphere of thought in which Professor Strasburger moves and works. Nor do we presume to question the propriety of such speculations as are here brought forward, especially on the ground which the author appears to take up, viz. that even where his speculations are of little permanent value they serve to direct attention to the points in question. Professor Strasburger undoubtedly shines more as an observer than as a theorist, however, and we cannot sympathise with him in many of his fine distinctions in this part of the book, *e.g.* the proposal to limit Naegeli's term "Micella," and the discussion as to the mode of increase of "microsomes," "Stärkebildner," &c.

We shall content ourselves with referring the reader to the book itself for the author's view on the "double refraction of organised structures," and on the "molecular structure of organised bodies," merely stating that he believes the phenomena of refraction by which Naegeli supports the intussusception hypothesis, can be equally well explained without assuming the presence of the Micellæ, as defined by Naegeli, as the effects of tension, &c.

Further, that organised bodies must be looked upon as constructed of extremely complex networks of molecular chains, nets, &c. Undoubtedly the discussion of this part of the subject is interesting, even fascinating, but we cannot avoid pointing out the danger of building general hypotheses on a foundation of considerations which are themselves so extremely hypothetical. This danger is, of course, clear to the distinguished author; but perhaps all his readers will not bear in mind that his object in publishing such speculations is rather to stimulate further work than to offer a solution of these great and difficult questions. We have by no means got to the bottom of the more immediate structure of organised structures: to go further and discuss their ultimate structure is, indeed, a work requiring careful treatment.

Strasburger's speculations are not confined to this subject however; he proceeds to an excellent criticism of recent views on the "Assimilation of carbohydrates," to which we cannot here do justice, and then concludes with speculations concerning the "*Rôle of the cell nucleus*," "The penetrability of the cell wall," and "The behaviour of the cell nucleus in fertilisation."

We have already referred to the idea that in Angiosperms the substance of the nuclei in the pollen grain becomes distributed, and after passage into the egg again aggregated. In cases where a single pollen tube is formed the nucleus can be traced to near the end, preserving its nuclear substance in this form till the last moments before passage over to the egg; in Gymnosperms it does not thus disappear, but may even multiply. That the nucleus can suffer "fragmentation" is proved by what occurs in *Chara foetida*, &c.

Strasburger withdraws his earlier statement, that the cell-nucleus in the mother-cells of spermatozoids in Ferns falls to pieces. As Schmitz says, the nucleus here enlarges until it has gathered all the protoplasm to itself, a process agreeing with what Fleming finds in the case of animals.

In higher organisms the male element becomes more and more reduced till little more than the nucleus remains, the tail of the spermatozoid perhaps being cell-plasma. Here, then, is some support for the view that the nucleus is the proteid former, or, at any rate, plays an important part in the formation of the basis of life.

It is interesting to note that in *Spirogyra*, where the nucleus was believed to disappear in copulation, Schmitz finds by reagents that it is in substance still there.

As to the *rôle* of the cell nucleus, Strasburger considers



that his lately published view admits of more support. It does not govern the division of the cell, as was formerly believed, and Schmitz came independently to the same idea as Strasburger, that the nucleus must have some important duty to perform in the regeneration or increase of proteids.

The nucleus remains in all cells which have to regenerate or increase their protoplasmic contents, and it disappears last in the living cell. Numerous examples are given, and the author here exercises abundantly his power of concentrating isolated individual discoveries to the support of his hypothesis; we must refer the reader to the book itself, however, for the numerous details in this connection.

As to the penetrability of the cell-wall, the author sets out by remarking that he has little beyond hypothesis to offer. His studies of the cases where protoplasm passes from cell to cell through tüpfel, canals, &c., in the walls, and comparisons of what others have done, lead him to believe that the protoplasm of the cells in a plant may be continuous by means of extremely numerous fine filaments even where too delicate to be observed. It is in such connections as this that Prof. Strasburger exhibits to the full his daring and ingenuity in the wide field of hypothesis. As we have shortly indicated, one fifth of the present book consists in great part of brilliant speculations. If the author were not so well known as an indefatigable and accurate observer, such attempts to extend our sphere of knowledge by hypothesis would probably receive less attention than they deserve; as it is, no biologist will undervalue the wildest thoughts of the author of 'Zell-bildung und Zell-theilung,' and we can honestly welcome the present book as a valuable acquisition to the literature of the cell theory, perhaps congratulating ourselves that the composition and literary ability displayed do not constitute the most forcible battery of Prof. Strasburger's logical artillery.

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*Recent Researches on the Cilio-Flagellata.* By PROFESSOR  
T. JEFFERY PARKER.

THIS interesting group of Infusoria has lately received the attention of a Danish zoologist, Mr. R. S. Bergh, who publishes an elaborate monograph on the subject in the last number of Gegenbaur's 'Morphologisches Jahrbuch.'<sup>1</sup>

<sup>1</sup> R. S. Bergh "Der Organismus der Cilioflagellaten, eine phylogenetische Studie." 'Morph. Jahrb.,' Bd. vii, 2 Heft, pp. 177-288, pl. xii-xvi.



The Cilio-flagellata are intermediate in characters between the flagellate and ciliate Infusoria. Like the Flagellata, they possess, as their chief organ of locomotion, a long whiplash-like cilium or flagellum, in addition to which they are provided, like the Ciliata, with ordinary small vibratile cilia, usually restricted to an incomplete annular band round the body.

The form of the body is always bilaterally asymmetrical; that is, there is a clear distinction between dorsal and ventral aspects, anterior and posterior ends, and right and left sides; but the two latter never resemble one another perfectly, the body being divisible into two unequal and dissimilar portions by a median vertical plane. The variations in the form of the body are very great; it may be compressed from before backwards, or from above downwards, or from side to side, and may be produced into remarkable horn-like processes, which are characteristic of particular genera.

Except in three genera, the body is provided with an exoskeleton, in the form of a membrane, which may be either structureless or variously ornamented. On the ventral aspect there is, in most genera, either a large aperture or a longitudinal slit in the membrane, through which the protoplasm comes into direct contact with the exterior. There is also usually a transverse groove, through apertures in which the cilia are protruded.

But the point of chief interest in the skeleton is its chemical composition. Bergh has succeeded in proving, by numerous chemical tests applied to a large number of species, that the membrane consists of cellulose, or at any rate of some very similar carbo-hydrate. This, I believe, is the first time that cellulose has been actually demonstrated in the cell-wall of the Protozoa, the only case in which that substance has hitherto been known in the animal kingdom being that of the Tunicata.

Equally important are the results of the investigation of the contained protoplasm of these organisms. It is usually divided into ectoplasm and entoplasm, the latter of which is found to contain chlorophyll, diatomin (the yellowish-brown colouring matter of diatoms), and starch. Chlorophyll is already known to occur in many animals of widely-separated groups, starch has hitherto been proved to exist only in the green Turbellarians, and diatomin has never before been known out of the vegetable kingdom.

Professor Huxley says, speaking of the differences between animals and plants,<sup>1</sup> "The most characteristic morpho-

<sup>1</sup> 'Anatomy of Invert, Animals,' p. 45.

logical peculiarity of the plant is the investment of each of its component cells by a sac, the walls of which contain cellulose or some closely analogous compound; and the most characteristic physiological peculiarity of the plant is its power of manufacturing protein from chemical compounds of a less complex nature. The most characteristic morphological peculiarity of the animal is the absence of any such cellulose investment. The most characteristic physiological peculiarity of the animal is its want of power to manufacture protein out of simpler compounds."

It will be seen that both these distinctions break down in the case of the Cilio-flagellata; their cell-wall is proved—as Huxley suggested might be the case, in a note to the passage just quoted—to be practically identical with that of plants, and the presence of starch proves clearly that the chlorophyll has the same function as that of plants, the decomposition of the carbonic acid in the surrounding medium. Bergh, indeed, believes that in many genera the nutrition is entirely like that of a plant, and that no solid nutriment is ever taken up; and great weight must be attached to an opinion founded on so many careful observations, though I must confess that the ventral aperture in the test becomes somewhat inexplicable if it is not to be looked upon as an ingestive area. Still, there can be no doubt that if the Cilio-flagellata were an isolated group, Bergh's researches would oblige us to consider many of them as indubitable plants, and it is only comparative morphology which forbids this view of their affinities. They are so closely allied, on the one hand, to the Flagellata, many of which possess the most undoubted animal characteristics, and on the other, to the Ciliata, which no one would dream of considering as plants, that their systematic position must remain unaltered, and they must simply be taken as another and very striking instance of the impossibility of drawing anything like a hard-and-fast line between the animal and vegetable kingdoms.

As to the systematic arrangement of the group, Bergh divides it into two families, one of which—the *Adinida*—contains a single new genus and species, *Prorocentrum micans*; while the other—the *Dinifera*—contains three sub-families and eleven genera.

*Prorocentrum*, discovered by Bergh, is interesting as forming the nearest ally of the group to the Flagellata. It has an oval compressed body, with both flagellum and cilia at the anterior end, and possesses neither transverse nor longitudinal grooves. Its membrane consists of two valve-like moieties.

Among the *Dinifera*, the first sub-family, *Dinophyida* approaches most nearly to *Adinida*, having the transverse groove near the anterior end. The second sub-family, *Perididnida*, contains the typical genera, *Peridinium*, *Glenodinium*, *Ceratium*, &c., and is distinguished by having the transverse groove about the middle of the body. The third and last sub-family, *Gymnodinida*, contains three genera, distinguished by the entire absence of a membrane.

It is these naked genera which approach, in a remarkable way, to the ciliata. One species of *Gymnodinium*, for instance, exhibits in the ectoplasm the curious muscle-like myophan-striae so characteristic of *Paramœcium*, *Spirostomum*, and other ciliate forms. The curious *Polykrikos* was considered by Uljanin as a Turbellarian larva, and by Bütschli was placed among the Ciliata, in spite of its long flagellum. Bergh considers it as a true cilio flagellate, distinguished by having several instead of one transverse ciliated grooves. It is also remarkable for possessing trichocysts, which, as figured by Bergh, have the closest resemblance to the thread-cells of Cœlenterata.

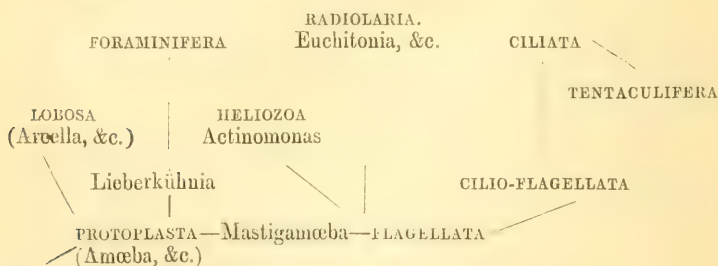
I cannot but think it a matter for regret that Bergh should be so permeated with "Haeckelismus" as to adopt the custom of calling his classification a phylogeny. The method of showing affinities by genealogical trees or other diagrams is a convenient and commendable one, but to call a natural arrangement of a group, based upon the study of recent forms only, a phylogeny, is a misuse of language, and gives a fallacious appearance of certainty to what is, at the best, only well-founded conjecture.

One point of great interest in regard to several of the genera is their excessive variability. Bergh's object having been to make a thorough investigation of the group, and not merely to discover new species, he has paid great attention to the varieties of each species, and has succeeded in showing, as Carpenter, W. K. Parker, and Rupert Jones, did for the Foraminifera and Haeckel for sponges, that each species consists of a "form-cycle" of individuals, differing so much that the extremes of the series would be ranked, without hesitation, as distinct species, if the intermediate steps were unknown.

It is from some such form as *Gymnodinium* that Bergh considers the Ciliata to have been derived, the *Peritricha* (*Vorticella*, &c.) being, according to him, the oldest and least modified subdivision of the group. The interesting genus *Mesodinium* is in many ways intermediate between the Cilio-flagellata and the Peritricha. It has an equatorial

band of cilia situated in a transverse furrow, but it is devoid of a flagellum, and possesses a mouth and temporary anus. From the position of the mouth Bergh considers that the anterior pole of a ciliate answers to the posterior pole of a cilio-flagellate or flagellate infusor.

One theory of more general interest is advanced, namely, that the Flagellata are the most primitive of Protozoa, and "form a starting-point from which the Noctilucae, the Rhizopoda, the Cilio-flagellata, and through these the Peritricha, have developed." The main argument for this view is that so many of the Rhizopoda begin life as mastigopods or flagellate forms. One cannot but think, however, that this is making too much of embryological evidence. *A priori*, it entirely seems more likely that a flagellum should have arisen as a differentiated pseudopod than that a pseudopod should have arisen as a degenerated flagellum; but the evidence is altogether too scanty for any very consistent theory to be built upon it. At present it seems to me to be impossible to say whether the myxopod or the mastigopod should be considered as phylogenetically the older; and I think, therefore, that the following scheme, devised for my last year's lectures, expresses the relationships of the groups of Protozoa as correctly as the evidence now at our disposal will enable us to do:



The monera of Haeckel are not included in this scheme, but as these can hardly be considered without further investigation to form a natural assemblage, since they are united upon a single negative character, I think it best to leave them out of consideration for the present.



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Fig. 1.

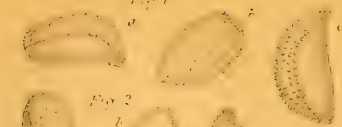


Fig. 2.



Fig. 3.



Fig. 4.

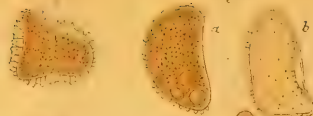


Fig. 5.



Fig. 6.



Fig. 7.

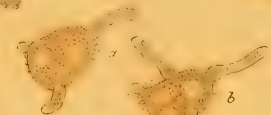


Fig. 8.



Fig. 9.

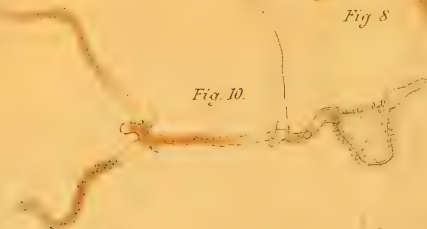


Fig. 10.



Fig. 11.



Fig. 12.

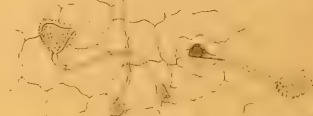


Fig. 13.



Fig. 14.



Fig. 15.



Fig. 16.





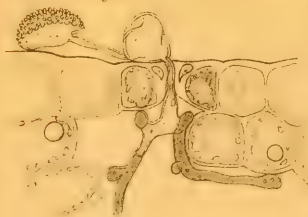
*Fig 19*



*Fig 18*



*Fig 20*



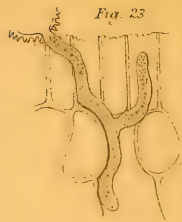
*Fig 21*



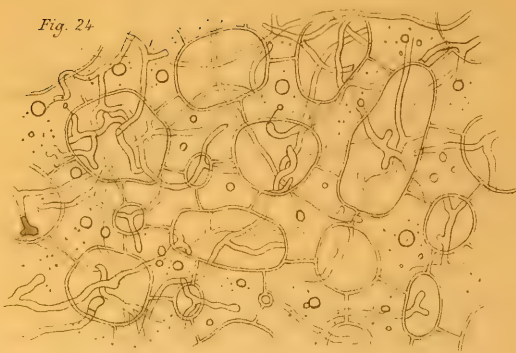
*Fig 31*



*Fig 23*



*Fig 24*



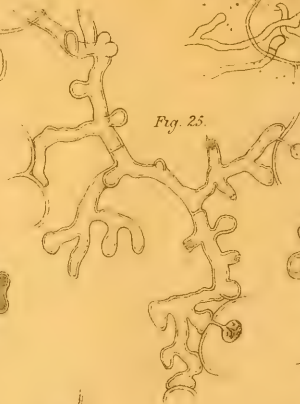
*Fig 22*



*Fig 26*



*Fig 25*



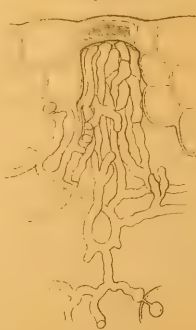
*Fig 27*



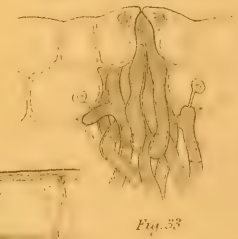
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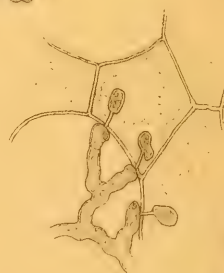
*Fig 32*



*Fig 33*



*Fig 29*



*Fig 30*

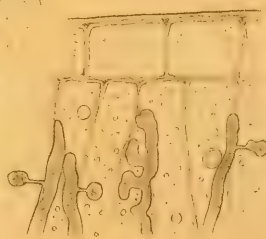








Fig. 34



Fig. 35



Fig. 36



Fig. 37

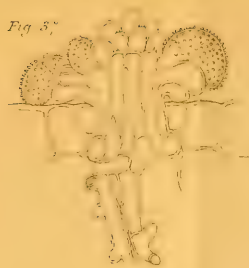


Fig. 38



Fig. 39



Fig. 42



a



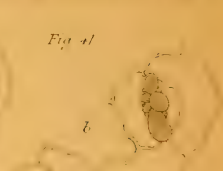
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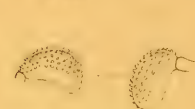
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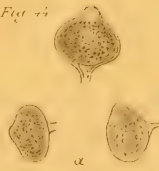


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Fig. 41



a

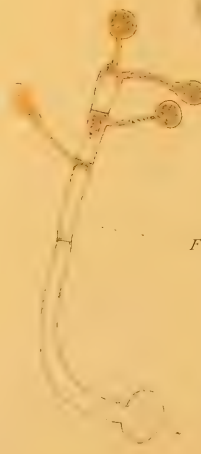
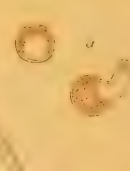
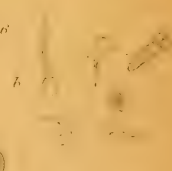


Fig. 45



a

Fig. 46



b



c



d



## JOURNAL OF MICROSCOPICAL SCIENCE.

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### EXPLANATION OF PLATES I, II, & III,

Illustrating Mr. Marshall Ward's Paper on *Hemileia vastatrix*.

FIGS. 1—3.—A uredospore detached in water, and viewed in various positions by rolling: the contents are omitted for simplicity. Zeiss J., oc. 4. Fig. 1, *a*, *c*, and Fig. 2 *a*, are seen from the side; Fig. 1 *b*, from above; Fig. 2 *b*, from below; Fig. 3, from the end by which spore would be attached.

FIG. 4.—Similar spore seen in (oblique) transverse optical section. Zeiss J., oc. 4.

FIG. 5.—A similar spore, with oily drops among the orange-coloured contents (*a*); after pressure these are driven out (*b*) and float free. Zeiss J., oc. 4.

FIG. 6.—A spore to which dilute glycerine was added: the withdrawal of water demonstrates the presence of the endospore (*a*). Careful washing causes the gradual resumption of original state (*b* and *c*). The spore is viewed obliquely from one end. Zeiss E., oc. 4.

*d*, piece of exospore in section. Zeiss J., oc. 5.

FIG. 7.—Uredospores which have become filled with vacuoles after lying in water for several hours. In series *a* the vacuoles (i) disappeared on adding (ii) weak iodine solution; no reappearance (iii) after washing. On addition (iv) of potash the contents become clearer, and the exospore swells. Series *b*, a similar spore before and after treatment with weak sugar solution: the vacuoles go, and the endospore contracts away from exospore. In *c*, are similar results obtained by adding sodium chloride solution, the endospore becomes wrinkled and contracted. *d*, vacuolated spore before and after treatment with weak potash. All Zeiss E., oc. 4.

*e*, similar fresh spores more magnified. All optical sections. Zeiss J., oc. 5.

FIG. 8.—Uredospores at the commencement of germination (in water): the endospore protrudes at three or four points, forming delicate tubes into which the coloured granular contents slowly pass. The clearer spaces are vacuoles (sap cavities); a beautiful streaming motion is often seen in the germinal tubes. *a* had been sown twelve hours; *b*, the same spore, five hours later. Zeiss J., oc. 4.

FIG. 9.—Similar preparations: *a*, after eighteen hours in water; *b*, same after twenty-two hours; *c*, same after thirty hours. All Zeiss J., oc. 4.

FIG. 10.—A uredospore after twenty-four hours' germination in water. Zeiss J., oc. 4.

FIG. 11.—Two similar objects after thirty hours' germination in water. Zeiss E., oc. 4.

FIG. 12.—Uredospore after about forty hours' germination in water: he curved, branched, and even-coiled germinal tube forms a pyriform swelling at apex, into which the contents pass. Zeiss D., oc. 4.

# JOURNAL OF MICROSCOPICAL SCIENCE.

## EXPLANATION OF PLATE IV,

Illustrating Mr. K. Mitsukuri's Paper on the "Development of the Suprarenal Bodies in Mammalia."

### *Explanation of Figures.*

The outlines of all the figures, except Fig. 10, are drawn with Zeiss's obj. A and camera lucida with eye-piece 4, and then reduced one-third, except Fig. 1. Fig. 10 is drawn with Zeiss's obj. a and the same camera lucida.

### *General Letters of Reference.*

*ao.* Aorta. *c.* Cortical part of the suprarenal bodies. *ch.* Notochord. *m.* Medullary part of the suprarenal. *n.* Peripheral sympathetic part with ganglion cells. *p.* Peripheral sympathetic part without ganglion cells. *p.p.* Body cavity. *s.r.* Suprarenal bodies. *symp.* Sympathetic cords. *v.* Veins. *v.c.* Cardinal veins. *v.c.i.* Vena cava inferior. *W.b.* Wolfian body.

FIG. 1.—A part of the transverse section of the adult suprarenal body of the rabbit.

*d.* Capsule. *a.* The outermost zone of the cortex. *b.* Zona fasciculata. *c.* Zona reticulatis. *w.w.* Blood-cavities.

FIG. 2.—A longitudinal section of the posterior end of the adult suprarenal body of the rabbit. The upper end of the figure is posterior.

*a.* Cells like ganglion cells. *b.* A place where one of the irregular cords of cells taper and seem to pass off into nerve fibres. *d.* Blood-capillaries. *g.* Ganglion-like mass. *i.* Blood-vessel. *y.* Nerve-bundle.

FIG. 3.—Section from a rabbit embryo twelve days old.

FIG. 4.—Section from a rabbit embryo fourteen days old.  
*g.* Germinal band.

FIG. 5.—Section from a rabbit embryo sixteen days old.

*a.* Nervous fibres given off from the mass *n.*

FIG. 6.—Section immediately behind that represented in Fig. 5 from the same embryo.

FIG. 7.—Section of the right suprarenal from a rat embryo 23 mm. long.

*b.* Nervous bundles entering the suprarenal.

FIG. 8.—Section of the suprarenal from an embryo rabbit twenty-six days old, near the middle of the organ.

FIG. 9.—Ditto, near the posterior end. The outer part of the suprarenal is torn away.

*a.* Nervous bundle.

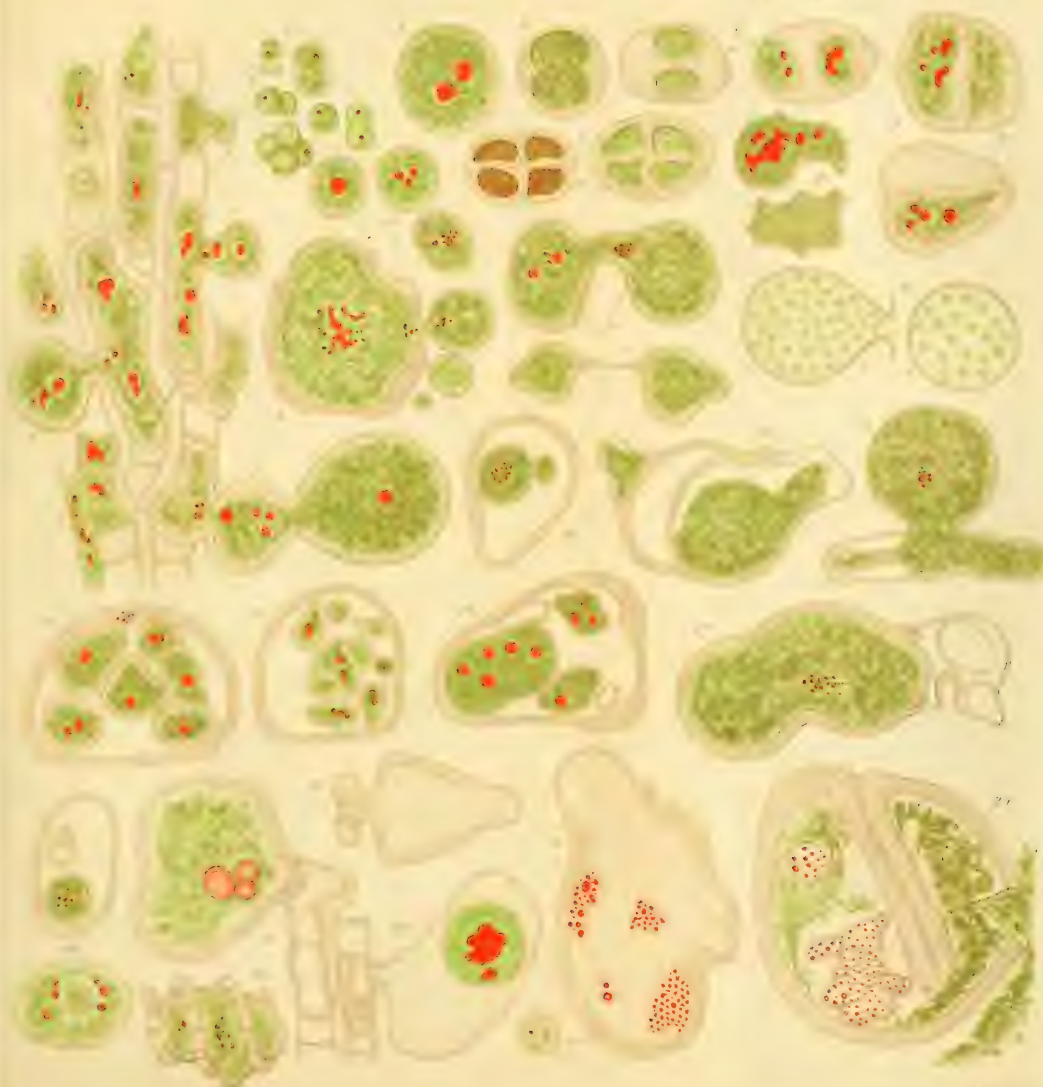
FIG. 10.—Series of diagrammatic longitudinal sections of the suprarenal from an embryo rabbit twenty-four days old.

A is the furthest from and D the nearest to the median axis of the body of the embryo. Between A and B there is one section. B and C are consecutive. Between C and D there are two sections. In each figure the upper end is anterior and the right side dorsal.













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## EXPLANATION OF PLATE V,

Illustrating Mr. Geddes' "Observations on the Resting Stage of *Chlamydomyxa labyrinthoides*, Archer."

*Magnified 200 to 350 diameters.*

FIG. 1.—Various stages of the development of *Chlamydomyxa*. *a*, *Protococcus* form; *b*, the same elongating; *c*, a more advanced stage; *d* and *e*, these bursting through; *f*, an adult form, from which a new outflow has arisen.

FIG. 2 *a*.—*Protococcus* forms; 2 *b*, larger ditto.

FIG. 3.—Large *Protococcus* form, remaining undivided.

FIG. 4.—Another, divided into two equal and similar portions.

FIG. 5 *a* and *b*.—Others divided into four.

FIG. 6 *a* and *b*.—Cysts, within which two new equal and similar cysts have formed.

FIG. 7 *a*.—Cyst divided by a transverse partition into two approximately equal halves; *b*, another of the same, from one chamber of which the contents have disappeared.

FIG. 8.—Large adult *Chlamydomyxa*, from which a new outflow is taking place. The adjacent smaller form may have arisen in the same way, and become closed off.

FIG. 9 *a*.—Case of outflow, where the new thin-walled portion is almost of equal size to the parent mass; *b*, case in which the two portions are only connected by a thread of cellulose.

FIG. 10.—Two masses of peculiar form, which appear just to be leaving the amœboid state, and developing new cellulose walls. Probably these have wandered from cysts.

FIG. 11 *a*, *b*.—Two cases in which the chlorophyll is not uniformly diffused throughout the whole or great part of the protoplasm-granules, as is generally the case, but is collected into definite patches at tolerably regular distances.

FIG. 12.—Case in which new cellulose wall has been formed at one side within but free from the former wall, at a pause in the outflow.

FIG. 13.—Example showing a morsel of protoplasm, separated at an early period; a subsequent outflow of the whole protoplasm, with deposition of new cell wall, followed by two different periods of shrinkage, as demonstrated by the existence of two internal strata of cellulose; the older distinct near apex, the inner and younger still incipient, indicated by a single line.

FIG. 14.—Specimen containing six young cysts, all nearly equal and similar.

FIG. 15.—Another specimen, containing eight or nine young cysts, much less equal and similar.

## EXPLANATION OF PLATE V—*continued*.

FIG. 16.—Case in which young cysts are very unequal and dissimilar.

FIG. 17.—Specimen which appears to have originally contained four cysts, of which three are empty, their contents having apparently wandered off, while the fourth has resumed growth within the former limiting wall. Two of the empty cysts are united to the parent wall.

FIG. 18.—Specimen containing two unequal cysts.

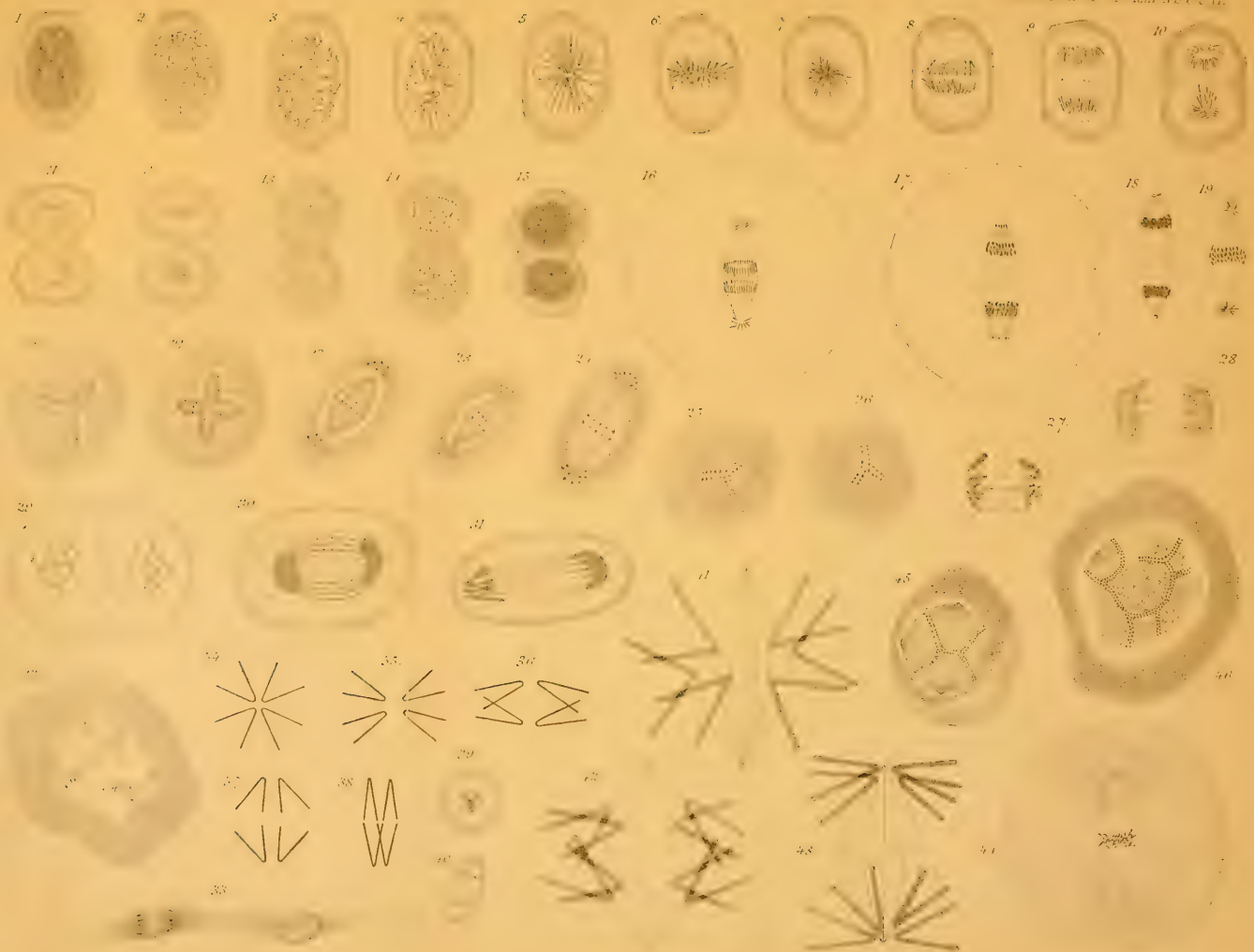
FIG. 19 *a*.—Another in which the coloured protoplasm has contracted itself into a sphere, leaving a smaller, almost empty, and colourless cyst free. *b*, specimen containing in its interior a peculiar double cyst, this time deeply coloured.

FIGS. 20, 21, and 22.—Specimens of which the history is recorded with extreme clearness in their cellulose walls. In Fig. 21 are to be seen small cysts, this time tinged red. Compare Figs. 19 *a*, 19 *b*, 23 *a*, 23 *b*.

FIG. 23 *a*.—Adult cyst, from which contents have been expressed, showing curious warts formed by the deposition of cellulose over particles of red colouring matter. *b*, the smallest form in which a wart was observed.

FIG. 24.—Adult cyst, containing warts of which the laminated structure is clearly visible. This individual has also enclosed at an early period a filamentous alga, round which it has deposited an extremely thick coating of cellulose.

FIG. 25.—Young *Chlamydomyxa* entwined by parasitic hypha.







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## DESCRIPTION OF PLATE VI,

Illustrating Mr. J. T. Cunningham's "Review of Recent Researches on Karyokinesis and Cell Division."

FIGS. 1—15.—Successive stages in the indirect (Karyokinetic) division of an animal cell (after Flemming). Each phase is supposed to have been fixed and stained.

Fig. 1. Resting nucleus, ground substance stained, nucleoli shown as thickened spots of the reticulum. Fig. 2. Convolution; in this, and all the figures up to 14th, only the fibrillar part of the nucleus is stained. Fig. 3. Wreath or garland. Fig. 4. Fibrils breaking up into "loops;" commencement of the mother star. Fig. 5. Mother star, diastole, fibres splitting longitudinally. Fig. 6. Systole of the star. Fig. 7. Return to diastole, the splitting complete. Fig. 8. Equatorial plate. Fig. 9. Basket form of the daughter nuclei; indications of the achromatic spindle and of the cell plate shown. Fig. 10. Dyaster. Fig. 11. Wreath form of the daughter nuclei. In the following figures the daughter nuclei pass into the resting state. In Fig. 15 the ground substance is again stainable.

FIGS. 16, 17, 18, and 19.—From figures given by Flemming from preparations of egg of *Toxopneustes lividus*, presented to him by H. Fol.

FIGS. 20—26.—After Arnold ('Virchow's Archiv,' Bd. 78, 1879).

Figs. 20 and 21. From epithelioma of lower lip. Figs. 22—26. From carcinoma of mammary gland.

FIGS. 27—31.—Cartilage cells of larval Batrachia (After Schleicher, 'Archiv Mik. Anat.,' Bd. 16).

FIGS. 32 and 33.—From Eberth, 'Virchow's Archiv,' Bd. 67.

Fig. 32. Endothelium cell, from Descemet's membrane in frog. Fig. 33. Epidermis cell of rabbit, showing a "cell plate."

FIGS. 34—38.—Diagrams illustrating the process of division of the equatorial plate, according to Flemming.

Fig. 34. Diastole. Fig. 35. Systole of the mother star.

FIGS. 39 and 40.—From Dr. Walstein's preparations.

Fig. 39. Triradiate arrangement of chromatin.

FIGS. 41, 42, and 43.—Diagrams of the structure of karyokinetic figures in the cells of Salamandra, from Flemming's 'Beiträge,' iii Theil ('Arch. f. Mik. Anat.,' Bd. xx).

Fig. 41. Mother star. Fig. 42. Equatorial plate. Fig. 43. Daughter stars.

FIG. 44. First division of the segmentation nucleus in the fertilised ovum of *Sphærechinus brevispinosus*. Compressed star form of the chromatic figure (from Flemming's 'Beiträge,' Theil iii).

FIGS. 45 and 46.—Multiple division of the nucleus in cells of carcinoma.

Fig. 45 shows division into four, Fig. 46 into seven daughter nuclei.

Fig. 46 shows spindle (convolution) and nuclear plate coexisting in the same stage (from W. A. Martin's paper in 'Virchow's Arch.,' Bd. 86).

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### DESCRIPTION OF PLATE VII,

Illustrating Mr. G. F. Dowdeswell's Paper on "The Micro-Organisms which occur in Septicæmia."

FIG. 1 represents a section of the lungs of a septicæmic mouse, deeply stained with methyl aniline violet, and treated with a solution of sodic carbonate.

A shows a large vein in longitudinal section, in which amongst the red blood-corpuscles numerous deeply stained white corpuscles are seen; these are full of the minute Bacilli described in the text, which are also found free on the inner surface of the walls of the vessel. *b b* are capillary blood-vessels, seen in transverse section, in which some few of the same bodies occur. The tissues, in making the preparation, have separated from the walls of the vessel. The magnification is about 160 diam.

FIGS. 2 and 3 show the relative size of the organisms described, sketched by the camera lucida and magnified 2800 diam.

The small rods in Fig. 2 are the Bacilli first described in the text; their length is about  $\frac{1}{15000}$ th in. In the lower part of the figure are shown portions of an organism also described as identical with the *B. anthracis* or hay Bacillus. In the centre are given three red blood-corpuscles of the mouse; their size is somewhat less than those of man, viz. about  $\frac{1}{4000}$ th in.

FIG. 3 shows the most minute form of Bacillus described, the *B. septicæmiæ* of Koch; both the rods free as they appear on the walls of the vessels, and agglomerated in masses within the white blood-corpuscles.

Fig 1



Fig 2



Fig 3

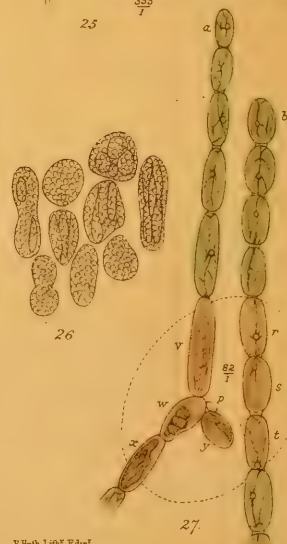
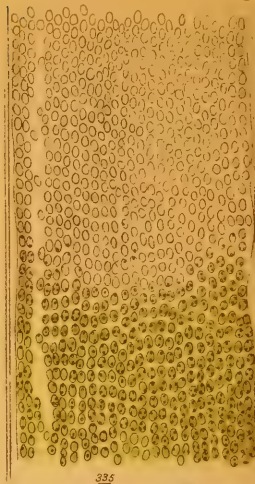
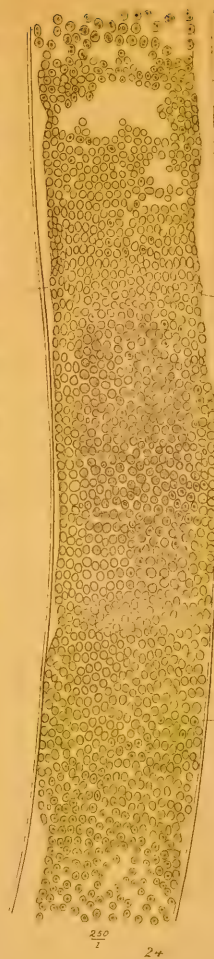
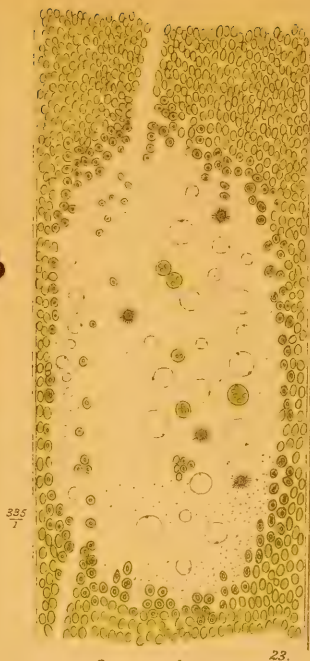
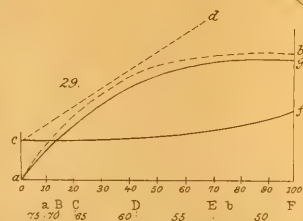
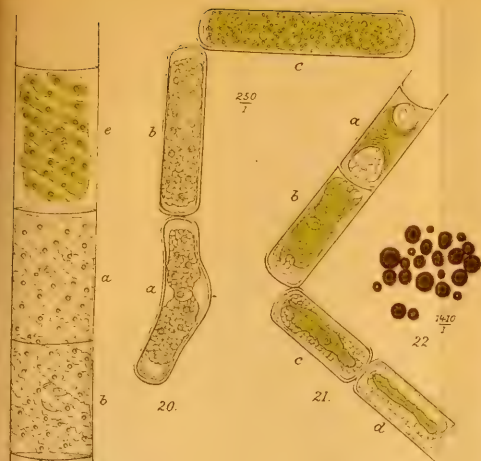
















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### EXPLANATION OF PLATES VIII & IX.

#### Illustrating Professor Bayley Balfour's Translation of Pringsheim's Researches on Chlorophyll.

FIGS. 1 and 2.—*Vallisneria spiralis*.

1. Chlorophyll-corpuscles after lying six days in dilute hydrochloric acid and being steamed for eight hours. 2. Hypochlorin-formation on chlorophyll-corpuscles; *a*, mass with firmer embedded pieces; *b*, nest with pointed prolongations.

FIG. 3.—*Minium*. Burst chlorophyll-corpuscles after five minutes boiling.

FIG. 4.—*Taxus baccata*. Hypochlorin-formation on chlorophyll-corpuscles after twenty-four hours lying in dilute hydrochloric acid.

FIGS. 5—7. *Edogonium*, sp.

5. Hypochlorin-scales. 6. Hypochlorin-needle. 7. Hypochlorin-needles bleached. All highly magnified.

FIG. 8.—*Begonia*, sp. Hypochlorin-formation on chlorophyll-corpuscles after five months in dilute HCl.

FIG. 9.—*Draparnaldia*, sp. Hypochlorin-filaments near amyllum-bodies after four weeks in same acid.

FIGS. 10—12.—*Spirogyra crassa*.

10. Cell treated with dilute HCl.

11. Nuclei from insolated cells with protoplasm-threads and plasma-knots; *a*, threads with knots; *b*, central plasma with coating of vesicles and knots. Exp. 20 and 21.

12. Portion of warmed chlorophyll-band with oil-vacuoles near amyllum-bodies.

FIGS. 13—18.—*Spirogyra*, sp.

13. Oil-vacuoles on bands after heating.

14. Protoplasm-threads dilating around amyllum-bodies.

15. Wavy rods of hypochlorin after twenty-eight hours lying in dilute HCl.

16. Hypochlorin-needles after eight days in same acid.

17. Isolated fragments of chlorophyll-bands with nests of hypochlorin-needles and rods.

18. Cells *c* and *d* insolated; whole filament subsequently stained with iodine.

FIG. 19.—*Spirogyra jugalis*. Cells *a*, *b*, *c*, *d*, insolated for some minutes; whole filament subsequently stained with iodine in potassium iodide, and then excess of iodine washed out.

EXPLANATION OF PLATE VIII & IX—*continued*.

FIGS. 20—22.—*Mesocarpus scalaris*.

20. Cells *a*, *b*, insolated in blue light; *c*, non-insolated cell showing tannin-vesicles. Exp. 41.

21. Cells *a*, *b*, *c*, *d*, cells insolated in red. Exp. 43.

22. Isolated tannin-vesicles stained with Millon's reagent.

FIGS. 23—26.—*Nitella*.

23. Insolated area highly magnified. The small and large clear vesicles with one or two peripheral dark needle-points are contents of unknown character.

24. Insolated area from a terminal leaf-segment, highly magnified.

25. Portion of insolated and non-insolated area of a cell treated with dilute HCl.

26. Decolorised chlorophyll-corpuses, some containing starch. Very highly magnified.

FIGS. 27.—*Tradescantia virginica*. *a*, *b*, stamen hairs; *p*, pollen grain. Cells enclosed in dotted line insolated for five minutes. Exp. 48.

FIG. 28.—Spectra of chlorophyll in a green cell and of the different coloured lights employed compared with the solar spectrum.

FIG. 29. Schema showing relation of assimilation to respiration in tissues with and without chlorophyll in different intensities of day-brightness: direct sunlight = 100.

*a b*, probable assimilation-curve in green tissue without chlorophyll.

*a g*, assimilation-curve of green tissue with chlorophyll, assuming that the colouring matter diminishes the assimilation 20 per cent.

*c d*, respiration-curve of green tissue without chlorophyll.

*c f*, probable actual respiration-curve of green tissue with chlorophyll.



Fig. 1.

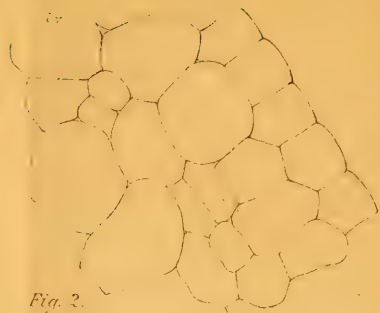


Fig. 2.

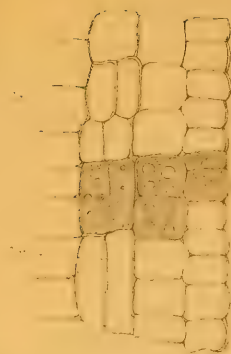


Fig. 3.



Fig. 9.

Fig. 5.

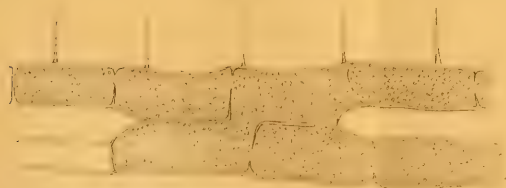


Fig. 7.

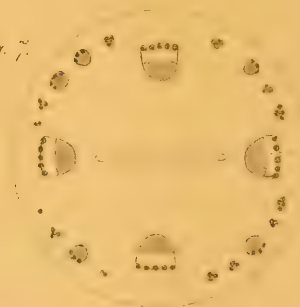


Fig. 6.

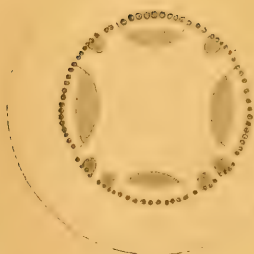
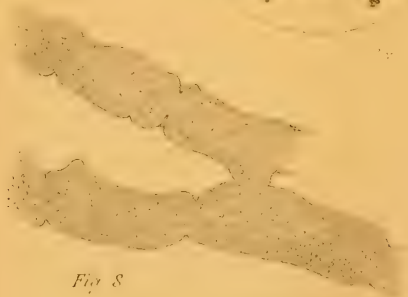


Fig. 4.



Fig. 8.







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## EXPLANATION OF PLATE X,

Illustrating Mr. D. Scott's Paper on "The Development of Articulated Laticiferous Vessels."

### *Explanation of the Figures.*

FIG. 1.—*Tragopogon eriospermus*. Diagrammatic cross-section of the hypocotyl.

*h. l. v.* Hypodermal laticiferous vessels. *ph. l. v.* Laticiferous vessels of the phloem. *ph.* Phloem part of fibro-vascular bundle. *xy.* Xylem part of ditto.

FIG. 2.—*Tragopogon*. Part of cross-section near base of cotyledons in dry seed.

*l. v.* Cells which form the hypodermal laticiferous vessels.

FIG. 3.—The same in longitudinal section, showing difference in the cell-contents.

*l. v.* As before.

FIG. 4.—Similar section from seed twenty-four hours after sowing.

FIG. 5.—Latex-vessels from the cotyledons of seedling, with root 1 cm. long. Absorption of cross-walls in progress.

FIG. 6.—*Scorzonera hispanica*. Diagrammatic cross-section of hypocotyl.

*l. v.* Laticiferous vessels. *ph.* Phloem. *xy.* Xylem.

FIG. 7.—Corresponding diagram of cotyledonary sheath.

Letters as before.

FIG. 8.—*Scorzonera*. Latex-vessels from base of young leaf, showing anastomosis and indications of the constituent cells.

FIG. 9.—Part of a latex-vessel from root of *Chelidonium majus*, showing thickened ridge.



Fig. 1.



Fig. 2.

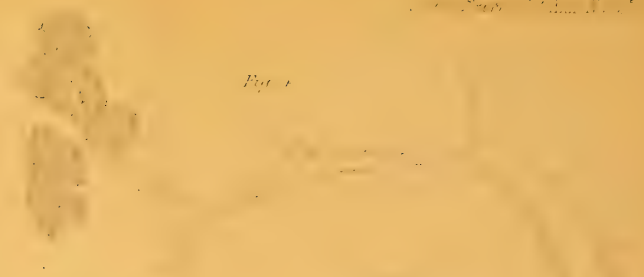


Fig. 3.



Fig. 4.

Fig. 5.



Fig. 6.



Fig. 7.

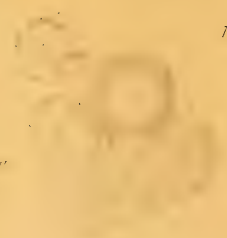


Fig. 8.



Fig. 9.



Fig. 10.

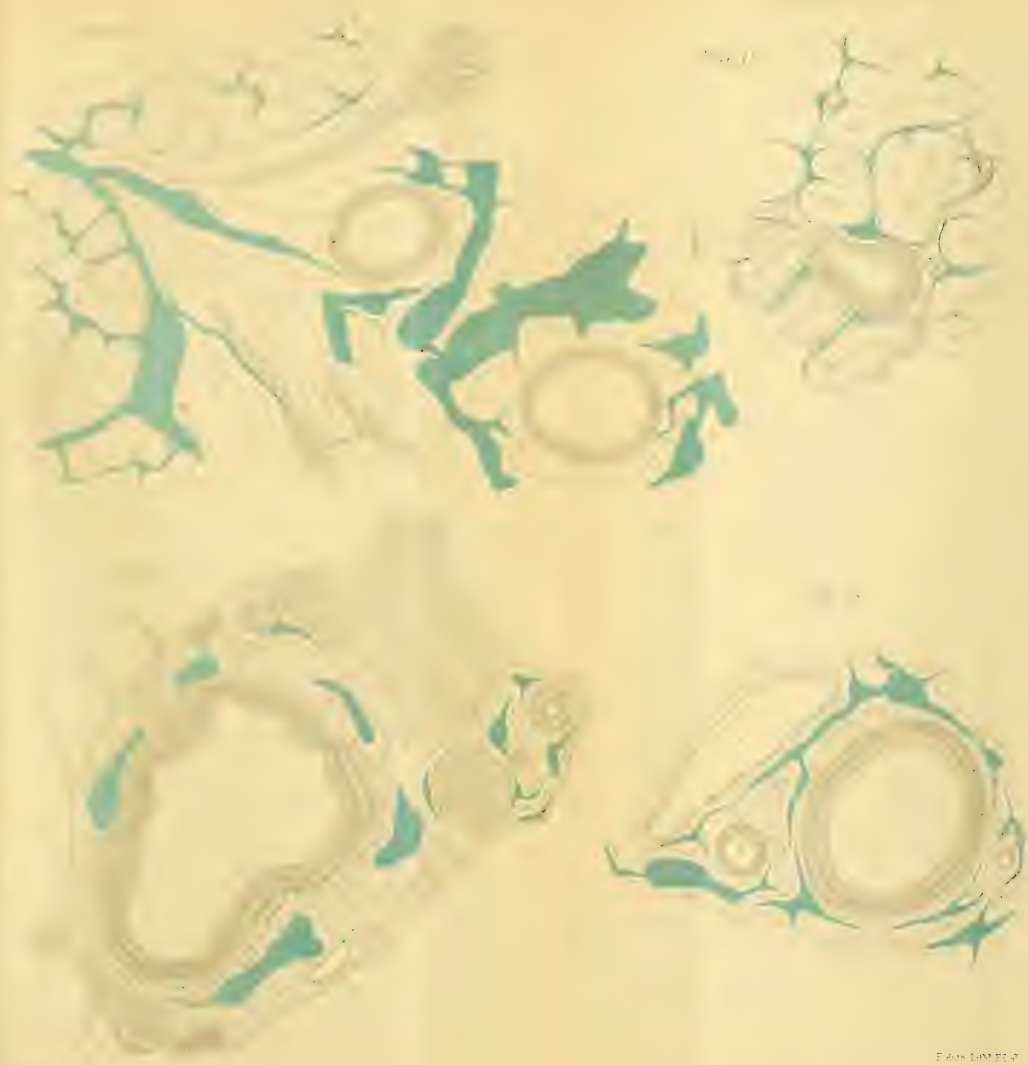


Fig. 11.











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## DESCRIPTION OF PLATES XI & XII,

Illustrating Dr. Klein's Paper "On the Lymphatic System and the Minute Structure of the Salivary glands and Pancreas."

FIG. 1.—From a section through the hardened parotid of the dog, showing the interlobular connective tissue after teasing it slightly out and staining it in logwood.

1. The fascicle plates; its constituent bundles of connective-tissue are cut transversely and obliquely. 2. Minute bundles passing between neighbouring plates. 3. Flattened connective tissue corpuscles seen in profile. Magnif. power about 350.

FIG. 2.—From a preparation of the interlobular connective tissue of the sublingual gland of the dog.

1. A fascicle-plate seen from the surface; the connective-tissue fibres are not represented. 2. Elastic fibrils. 3. A nucleus of a connective-tissue corpuscle seen in profile. 4. A coarsely granular plasma-cell. 5. Lymphoid cells flattened against one another. 6. Nuclei of connective-tissue corpuscles seen full face. Magnif. power about 250.

FIG. 3.—From the parotid of the dog, showing the transition of a minute intralobular duct into the intercalated or intermediate portion through the neck.

1. Intralobular duct. 2. Neck. 3. Intermediate part. Magnif. power about 350.

FIG. 4.—From a section through the parotid of the rabbit, showing the various changes the intralobular duct undergoes in structure on its transition into the alveoli.

At 1 the lining epithelium is columnar, and shows well the longitudinal fibrillation. At 2 the epithelium is polyhedral without any distinct fibrillation; the lumen of the duct is lined with a special inner nucleated membrane. This part corresponds to the "neck" of the duct. At 3 and 4 the epithelium consists of a layer of very transparent flat elongated cells with oblong nuclei. The inner lining membrane is very distinct. The two sections correspond to the intercalated or intermediary part. At 5 are the alveoli, cut in various directions and lined with columnar epithelial cells, showing well the intracellular reticulum. Magnif. power about 250.

FIG. 5.—From a section through the parotid of the dog, showing the tubular nature of the alveoli.

1. Wall of the alveoli seen from above. 2. Solid bridges between neighbouring alveoli. 3. Lumen. 4. An alveolus cut obliquely. The epithelial cells show very well the intracellular reticulum. Magnif. power about 350.

## PLATE XI & XII—*continued.*

FIG. 6.—An alveolus of the submaxillary gland of the guinea-pig.

1. The lining columnar epithelial cells show a distinction between an outer homogeneous or faintly striated zone, including the nucleus, and an inner more transparent zone, characteristic of the pancreas.

Magnif. power about 450.

FIG. 7.—From a section through the sublingual gland of the rabbit.

1. Intralobular duct in cross section. 2. Alveoli lined with mucous cells. 3. Groups of cells forming crescents. 4. Inter-alveolar connective tissue. Magnif. power about 250.

FIG. 8.—From a section through the sublingual gland of the dog.

1. Alveoli lined with ordinary columnar cells with distinct longitudinal fibrillation. 2. Alveoli lined with transparent mucous cells. 3. Inter-alveolar lymphoid corpuscles. Magnif. power about 350.

FIG. 9.—Transverse section through the sublingual gland of the guinea-pig.

1. Duct. 2. Transition of it into the alveoli. 3. Direct transition of the polyhedral epithelial cells of the duct into the columnar transparent mucous cells of the alveoli. 4. Alveoli whose epithelium is indicated in outline only. 5. The mucous cells show the intracellular reticulum. Magnif. power about 350.

FIG. 10.—From a section through the sublingual gland of the guinea-pig, showing a vein ensheathed in a lymphatic vessel.

1. Lumen of the lymphatic. 2. Lumen of the vein containing blood-corpuscles. 3. Endothelium of the lymphatic. 6 and 5. Adventitia of the lymphatic. 4. Muscular (middle) coat of the lymphatic. 7. A small blood-vessel cut across. 8. Endothelium covering the outer surface of the wall of the vein. 9. Endothelium lining the lumen of the vein. Magnif. power about 250.

FIG. 11.—*a* and *b* are two mucous cells of the submaxillary of the dog, the nuclei in open connection with the cell reticulum; *c*, two cells of the crescent, showing a dense cell reticulum. Magnif. power about 500.

FIG. 12.—From a section through the submaxillary gland of the dog; the lymphatics are injected with Berlin blue.

1. Interlobular ducts cut across. 2. Ganglia and nerve branches. 3. Lymphatics accompanying the ducts. 4. Lymphatics of the connective-tissue septa between the lobules. 5. Circum-alveolar lymphatics; the gland alveoli are shown in outline only. Magnif. power about 100.

FIG. 13.—From a section through the parotid of the dog; the lymphatics are injected with Berlin blue.

1. Large duct cut across. 2. Artery. 3. Vein. Magnif. power about 250.

FIG. 14.—From a section through the submaxillary gland of the dog; the lymphatics are injected with Berlin blue.

1. Intralobular duct cut across. 2. Alveoli cut in various directions; the lining epithelium is indicated. 3. The same, without the epithelium being indicated. Magnif. power about 150.

FIG. 15.—From a section through the sublingual gland of the rabbit; the lymphatics are injected with Berlin blue.

1. Cross section through the chief duct; in the fibrous coat of its wall are lymphatic vessels in section. 2. Nerve branches in cross section. 3. Nerve branches in cross section, but only indicated in outline. 4. The columnar epithelium composed of two layers. 5. Ganglia. 6. Veins. 7. Arteries. Magnif. power about 150.



*Ser. A1.*

*ep*

*Ser. B1.*

*hy*

*ep*

*gr*

*Ser. A2.*

*hy*

*ep*

*hy*

*gr*

*ep*

*Ser. B2.*

*pvs*

*gr*

*ep*

*hy*

*pvs*

*Ser. C1.*

*ep*

*pvs*

*hy*

*hy*

*Ser. B3.*

*yk*

*Ser. C2.*

*pvs*

*Ser. E1.*

*ep*

*yk*

*hy*

*yk*

*pvs*

*ep*

*hy*

*Fig. D.*

*yk*

*Ser. E2.*

*ep*

*gr*

*hy*

*m*

*hy*

*yk*



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## EXPLANATION OF PLATES XIII, XIV & XV,

Illustrating Messrs. Balfour and Deighton's Paper on "A Renewed Study of the Germinal Layers of the Chick."

N.B.—The series of sections are in all cases numbered from before backwards.

### *List of Reference Letters.*

*ep.* Epiblast. *hy.* Hypoblast. *m.* Mesoblast. *gr.* Germinal wall. *y/k.* Yolk of germinal wall. *pv s.* Primitive streak. *pr. g.* Primitive groove. *ch.* Notochord. *a. p.* Area pellucida. *o. p.* Area opaca.

### PLATE XIII.

SERIES A, 1 and 2.—Sections through the blastoderm before the appearance of primitive streak.

1. Section through anterior part of area pellucida in front of embryonic shield. The hypoblast here forms an imperfect layer. The figure represents about half the section. 2. Section through same blastoderm, in the region of the embryonic shield. Between the epiblast and hypoblast are a number of undifferentiated cells. The figure represents considerably more than half the section.

SERIES B, 1, 2 and 3.—Sections through a blastoderm with a very young primitive streak.

1. Section through the anterior part of the area pellucida in front of the primitive streak. 2. Section through about the middle of the primitive streak. 3. Section through the posterior part of the primitive streak.

SERIES C, 1 and 2.—Sections through a blastoderm with a young primitive streak.

1. Section through the front end of the primitive streak. 2. Section through the primitive streak, somewhat behind 1. Both figures show very clearly the difference in character between the cells of the epiblastic mesoblast of the primitive streak, and the more granular cells of the mesoblast derived from the hypoblast.

FIG. D.—Longitudinal section through the axial line of the primitive streak, and the part of the blastoderm in front of it, of an embryo duck with a well-developed primitive streak.

SERIES E,<sup>1</sup> 1, 2, 3 and 4.—Sections through blastoderm with a primitive streak, towards the end of the first stage.

1. Section through the anterior part of the area pellucida. 2. Section a little way behind 1 showing a forward growth of mesoblast from the primitive streak. 3. Section through primitive streak. 4. Section through posterior part of primitive streak, showing the great widening of primitive streak behind.

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<sup>1</sup> 3 and 4 of this series are placed on Plate XIV.

EXPLANATION OF PLATES XIII, XIV & XV—*continued*.

PLATE XIV.

SERIES F, 1 and 2.—Sections through a blastoderm with primitive groove.

1. Section showing a deep pit in front of primitive streak, probably an early indication of the neurenteric canal. 2. Section immediately following 1.

FIG. G.—Section through blastoderm with well developed primitive streak, showing an exceptionally deep slit-like primitive groove.

SERIES H, 1 and 2.—Sections through a blastoderm with a fully-developed primitive streak.

1. Section through the anterior part of area pellucida, showing the cubical granular hypoblast cells in this region. 2. Section slightly behind 1, showing the primitive hypoblast cells differentiated into stellate cells, which can hardly be resolved in the middle line into hypoblast and mesoblast.

SERIES I, 1, 2, 3, 4 and 5.—Sections through blastoderm somewhat older than Series H.

1. Section through area pellucida well in front of primitive streak. 2. Section through area pellucida just in front of primitive streak. 3. Section through the front end of primitive streak. 4. Section slightly behind 3. 5. Section slightly behind 4.

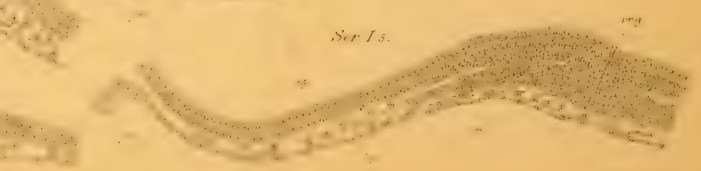
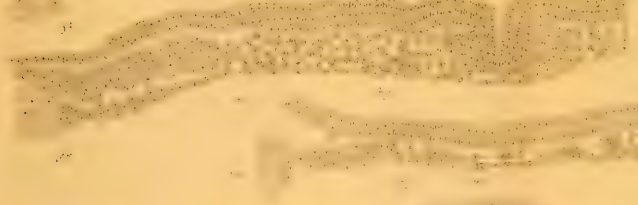
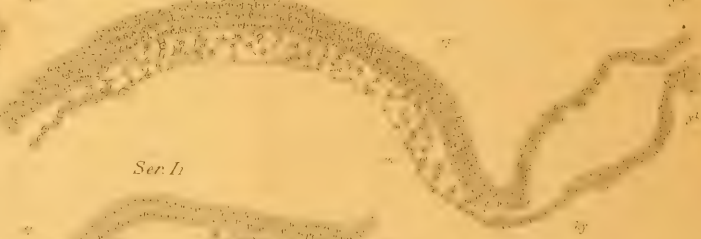
PLATE XV.

SERIES K, 1, 2, 3, 4 and 5.—Sections through a blastoderm in which the first trace of notochord and medullary groove have made their appearance. Rather more than half the section is represented in each figure, but the right half is represented in 1 and 3, and the left in 2 and 4.

1. Section through notochord immediately behind the head-fold. 2. Section showing medullary groove a little behind 1. 3. Section just in front of the primitive streak. 4 and 5. Sections through the front end of the primitive streak.

FIG. L.—Surface view of blastoderm with a very young primitive streak.





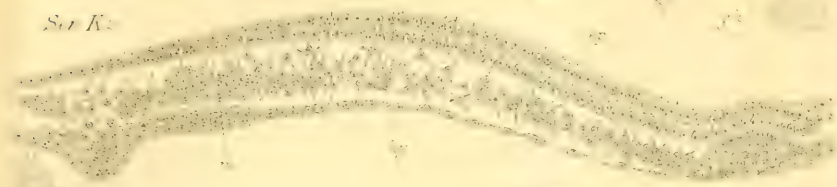


Ser. K<sub>1</sub>

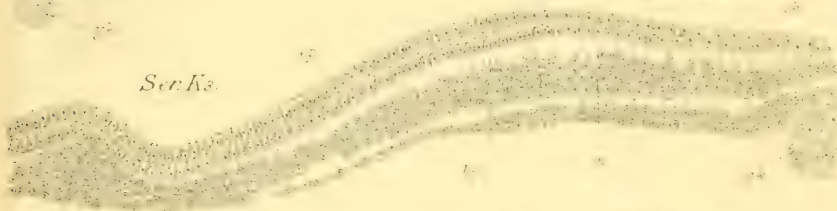
7



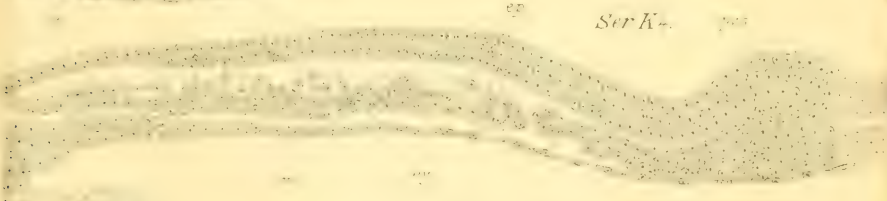
Ser. K<sub>2</sub>



Ser. K<sub>3</sub>



Ser. K<sub>4</sub>



Ser. K<sub>5</sub>



Fia. L.





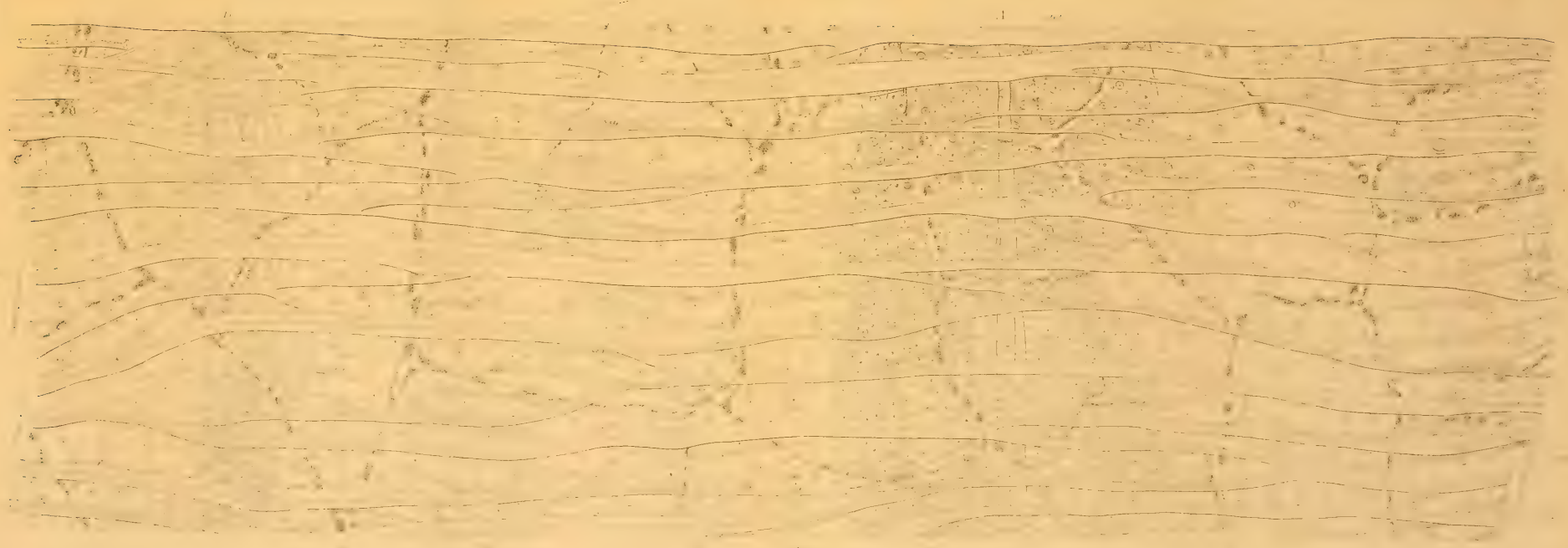




Fig 1



Fig 2





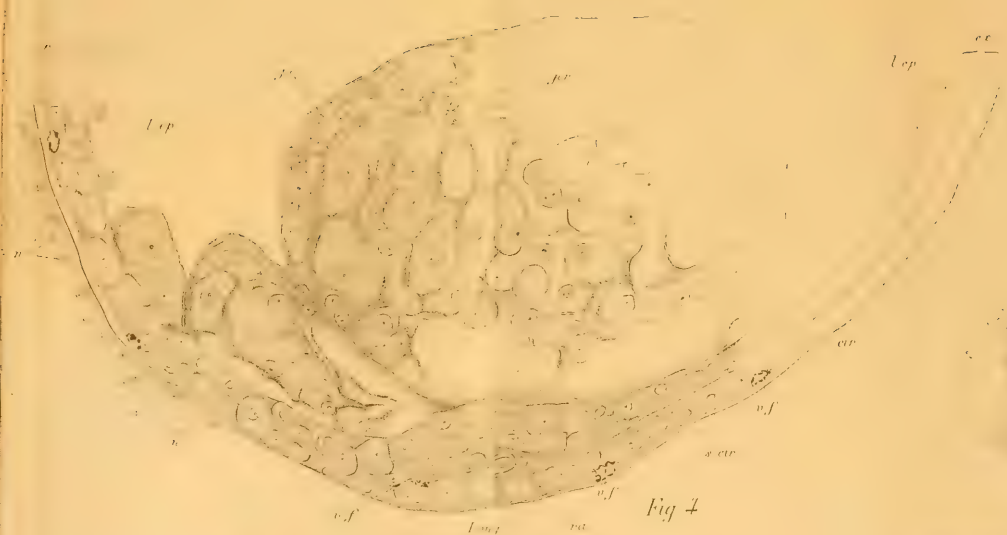


Fig. 4

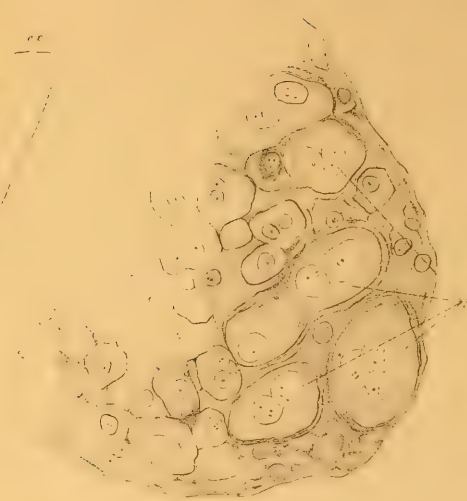


Fig. 5.



Fig. 6.

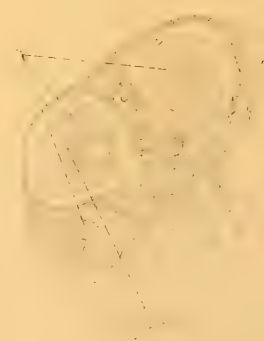


Fig. 9.



Fig. 10



Fig. 7.



Fig. 8





# JOURNAL OF MICROSCOPICAL SCIENCE.

## DESCRIPTION OF PLATES XVI, XVII, XVIII & XIX.

Illustrating Mr. Isao Iijima's Paper "On the Origin and Growth of the Eggs and Egg-strings in Nephelis, with some Observations on the 'Spiral Asters.'"

5—7 *g.*, 5th—7th ganglion. *A.* Rachal line. *B.* Mediad line. *cir.* Circular muscle-fibres. *ex.* External layer of the ovary-wall. *f. o.* Female orifice. *fol.* follicle. *g. c.* Germ-cells. *ger.* Germogen. *g. v.* Germinal vesicle. *l. ep.* Lining epithelium. *long.* Longitudinal muscle-fibres. *m. o.* Male orifice. *m. s.* Mediad side. *n.* Nucleus of the cellular tissue investing the ovaries. *ov.* Ova. *ova.* Ovary. *ra.* Rachis. *ra. s.* Rachal side. *s. cir.* Semi-circular muscle-fibres. *sp.* Spermatozoa. *st.* Egg-strings. *t.* Testis. *t. ov.* Termination of ovaries. *v. d.<sub>1</sub>.* Beginning of vasa deferentia. *v. d.<sub>2</sub>.* Coiled portion of ditto. *v. d.<sub>3</sub>.* That portion of ditto lying beneath the testicular sacs. *v. f.* Vaso-fibrous tissue. *y. fol.* Young follicle. *y. n.* Yolk-nucleus.

### PLATE XVI.

FIG. 1.—The reproductive organ *in situ*, magnified about three times.

FIG. 2.—The right ovarian tube drawn half-diagrammatically, showing the germogen and the egg-strings. Magnified about ten times.

FIG. 3.—A surface view of the ovary-wall, made out from both chromic acid and picro-sulphuric acid preparations. ( $\times 450$ .)

### PLATE XVII.

FIG. 4.—A cross-section of the ovary-wall, on the rachal side, showing different stages of the germogen. Picro-sulphuric acid. ( $\times 450$ .)

FIG. 5.—A cross-section of the germogen, a little more advanced in development than that of Fig. 4. Picro-sulphuric acid. ( $\times 450$ .)

FIG. 6.—A longitudinal section of an egg-string, somewhat schematically drawn. Magnified about twenty-five times.

FIG. 7.—A cross-section of an egg-string, in the plane of A—A of Fig. 6. Chromic acid. ( $\times 165$ .)

FIG. 8.—A cross-section of the same in the plane of B—B of Fig. 6. Chromic acid. ( $\times 165$ .)

FIG. 9.—A cross-section of the same at about c—c of Fig. 6. Chromic acid. ( $\times 165$ .)

FIG. 10.—The cross-section of the same at about d—d of Fig. 6. Chromic acid. (165.)

EXPLANATION PLATES XVI, XVII, XVIII & XIX—*continued.*

PLATE XVIII.

FIG. 11.—Longitudinal section of the ovary-wall, the germogen, and an egg-string near its smaller end. Chromic acid. ( $\times 165$ .)

FIG. 12.—A longitudinal section of an egg-string near its broad end. In one of the follicles an ova is established. Picro-sulphuric acid. ( $\times 450$ .)

FIG. 13.—The same as Fig. 12, showing different stages of the ovum. ( $\times 450$ .)

FIG. 14.—A longitudinal section near the middle of an egg-string. The larger ovum is nearly mature. Picro-sulphuric acid. ( $\times 450$ .)

PLATE XIX.

All preparations, except Fig. 19, were treated with acetic acid and clarified with a mixture of glycerine and potassic acetate.

FIG. 15.—An egg found floating in the ovarian fluid. ( $\times 450$ .)

FIG. 16.—Just after deposited, showing the "spiral asters." ( $\times 450$ .)

FIG. 17.—Optical section through the lower aster of Fig. 16, seen from above. ( $\times 450$ .)

FIG. 18.—Five minutes after laid. ( $\times 450$ .)

FIG. 19.—About the same stage as Fig. 18. Fresh condition. ( $\times 450$ .)

FIG. 20.—Three hours and a half after laid. ( $\times 450$ .)



Fig. 1

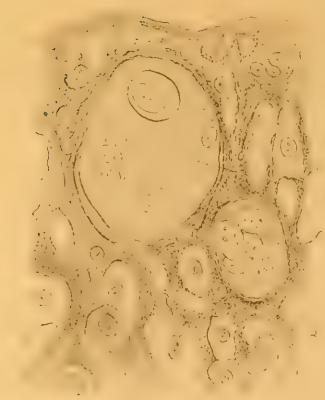


Fig. 2

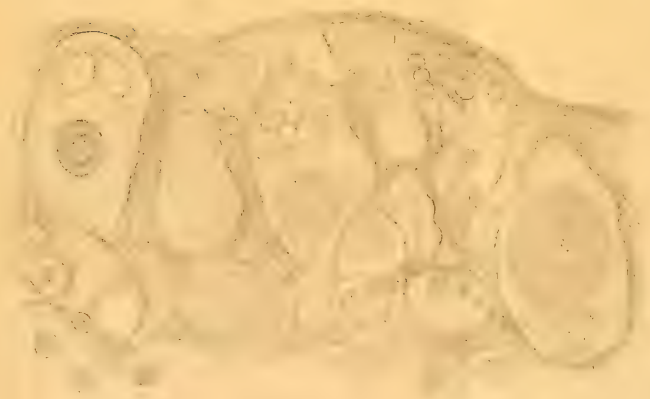


Fig. 3







Fig. 10

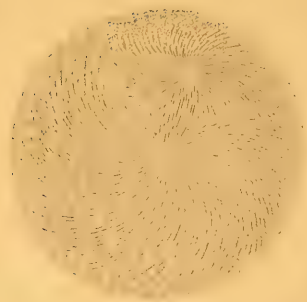


Fig. 11

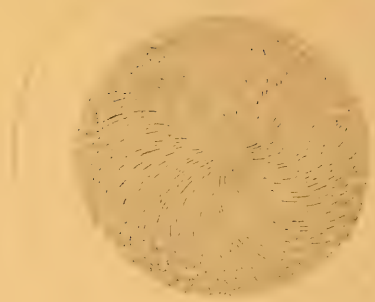


Fig. 12



Fig. 13

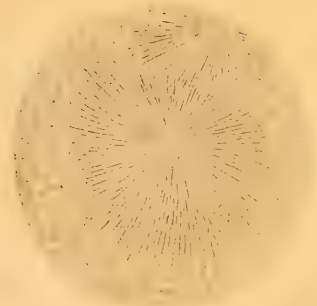


Fig. 14



Fig. 15







E. Ray Lankester del.

F. Roth Lith. Edin.



## JOURNAL OF MICROSCOPICAL SCIENCE.

### EXPLANATION OF PLATE XX,

Illustrating Professor E. Ray Lankester's Memoir on the  
"Chlorophyll-corpuscles and Amyloid Deposits of Spongilla and Hydra."

The following references are true for all the figures.

*n.* Nucleus. *c.* Normal chlorophyll-corpuscle. *cc.* Small and irregularly shaped chlorophyll-corpuscles. *ccc.* Unusually large chlorophyll-corpuscle. *g.* Colourless granules, probably related to the chlorophyll-corpuscles. *amv.* Amyloid substance occupying a large vacuole. *amg.* Amyloid granules, scattered in the superficial protoplasm of the cells of Spongilla. *br.* Irregular brown corpuscles of Hydra.

N.B.—A uniform green tint has been adopted in the plate for the corresponding green tint of the corpuscles; but it is to be remembered that often the corpuscles of Spongilla are of a more yellow green than here represented.

FIGS. 1 to 14 are from Spongilla. FIGS. 15 to 24 are from Hydra.

#### *Spongilla.*

FIG. 1.—Amœboid cell of *Spongilla fluviatilis*, showing nucleus (*n*), chlorophyll-corpuscles (*c*), colourless angular corpuscles (*g*), and large amyloid vacuole (*amv*). Living.

FIG. 2.—A similar cell, drawn from a living specimen (*ccc*), large chlorophyll-corpuscle with emarginated capsule.

FIG. 3.—Amyloid vacuole coloured purple, after treatment with dilute solution of iodine in KI.

FIG. 4.—Amœboid cell similarly treated, showing chlorophyll-corpuscles (*c*), amyloid granules (*amg*), and amyloid vacuole (*amv*).

FIG. 5.—Outline of an amœboid cell to show the superficial position of the amyloid granules, coloured purple by iodine.

FIG. 6.—Amœboid cell from a colourless specimen of Spongilla, showing nucleus (*n*) and (*g*) colourless angular corpuscles, the supposed representatives of chlorophyll-corpuscles; *gg*, a colourless corpuscle with cap-like development, similar in form to the chlorophyll-cap. Drawn from a fresh living specimen.

FIG. 7.—Similar specimen from a portion of Spongilla of a very pale green. It shows, besides the representative colourless corpuscles (*g*), three actual chlorophyll-corpuscles (*c*).

FIG. 8.—Amœboid cell of Spongilla, immediately after treatment with dilute iodine solution in KI, to show the superficially placed amyloid granules (*amg*).

## EXPLANATION OF PLATE XX.—Continued.

FIG. 9.—Isolated chlorophyll-corpuscles of *Spongilla fluviatilis*. *a*. Seen sideways to show the concavo-convex form of the chlorophyll-cap. *b*. Seen from the concave surface. *c*. Two chlorophyll-caps developed upon one corpuscle of colourless protoplasmic substance. *e*. and *f*. Chlorophyll-caps with adherent protoplasm. Diameter of the chlorophyll-caps averages  $\frac{1}{11000}$ th inch.

FIG. 10.—*a*. Corpuscle of dense colourless protoplasm, upon which six chlorophyll-caps have developed, of which five are visible. *b*. Colourless protoplasmic corpuscle and cap coloured green by chlorophyll.

FIG. 11.—Colourless corpuscles isolated from amœboid cells of a colourless *Spongilla*. They are more angular in form, and denser in substance, than the corpuscles upon which chlorophyll-caps are developed in green specimens.

FIG. 12.—Abnormal chlorophyll-corpuscle of *Spongilla*, of very large size ( $\frac{1}{5000}$ th inch in diameter). Chlorophyll has developed all over the surface of the corpuscle as a spherical envelope, whilst within are granules coloured also by chlorophyll. It is seen in optical section, and closely resembles the chlorophyll-corpuscles of *Hydra viridis*.

FIG. 13.—Amœboid cell of *Spongilla fluviatilis* fixed with osmic acid, and stained by picrocarmine.

FIG. 14.—Amœboid cell from colourless specimen of *Spongilla*, examined in October. It has been fixed with osmic acid, and stained with picrocarmine. Not only the nucleus (*n*) is stained, but the large amyloid vacuole (similar to that of figs. 1, 3, and 4) has taken a very intense carmine stain. The angular corpuscles (*g*) do not stain with carmine.

## *Hydra.*

FIG. 15.—Endoderm-cell of *Hydra viridis* isolated by teasing, fixed with osmic acid, and lightly stained with picrocarmine. It shows (*n*) nucleus, (*c*) chlorophyll-corpuscles, (*g*) irregular colourless bodies which stain faintly with carmine, (*tc*) an ingested thread-cell, (*cc*) minute rod-like chlorophyll-corpuscles.

FIG. 16.—Endoderm-cell of *Hydra fusca* isolated by teasing, fixed with osmic acid, and lightly stained with picrocarmine. It shows (*n*) nucleus, (*b*) "foot" of the cell, (*g*) irregular corpuscles, colourless, but with dark granules embedded in them. These appear to represent the chlorophyll-corpuscles of *H. viridis*, as do the corpuscles (*g*) in fig. 6, of colourless *Spongilla* represent the chlorophyll-corpuscles of green *Spongilla*.

FIG. 17.—Isolated chlorophyll-corpuscles of *Hydra viridis*. *a*. The spherical envelope of green-coloured substance is divided into three segments (surface view). *b*. Optical section of the same corpuscle, showing green-coloured granules lying in the colourless protoplasm, enclosed by the green envelope. *c*. Optical section of another corpuscle. *d*. An unusual form of corpuscle, devoid of superficial green-coloured envelope, but with numerous green-coloured granules embedded in the protoplasmic corpuscle. *e*. Optical section of another normal chlorophyll-corpuscle. Fresh, without reagents.

FIG. 18.—Portions of endoderm-cell of an olive-green coloured *Hydra*, found in association with *Hydra fusca*. These portions separated when the attempt was made to isolate endoderm-cells by teasing. Each contains irregular, angular, scattered and aggregated granules, coloured blue-green by chlorophyll. Fresh, without reagents.

FIG. 19.—Brown-coloured corpuscles, very frequently observed in endo-

## EXPLANATION OF PLATE XX.—*Continued.*

derm-cells of *Hydra viridis*, in association with the chlorophyll-corpuscles (see fig. 21). Fresh, without reagents.

FIG. 20.—*a.* to *l.* A number of examples of chlorophyll-corpuscles of *Hydra viridis*. All are seen in optical section excepting *c* and *g*, in which cases a surface view is also given. The green envelope is seen to be *incomplete* in the examples, *a, e, f, h, i*. The average diameter of these corpuscles is from  $\frac{1}{5000}$ th to  $\frac{1}{8000}$ th of an inch. Fresh, without reagents.

FIG. 21.—Portion of an endoderm-cell of *Hydra viridis*, showing normal chlorophyll-corpuscles (*c*), minute rod-like bodies coloured green (*cc*), and also peculiar brown corpuscles (*br*). Fresh, without reagents.

FIG. 22.—Spherical corpuscles isolated by teasing the endoderm-cells of an olive-green coloured specimen of *Hydra fusca*. All three contain angular granules set in the substance of the spherical corpuscle. In *a* and *b*, these were seen to be *colourless*, in *c* they were coloured *green*.

FIG. 23.—Normal chlorophyll-corpuscles of *Hydra viridis* from an endoderm-cell which had been fixed by dilute osmic acid, and subsequently treated with alcohol, so as to dissolve out the green colouring matter (chlorophyll). The cell was then subjected to a *prolonged* staining with picrocarmine. The part of the corpuscles originally coloured green by chlorophyll (chromophorous substance) does not stain, but the enclosed protoplasm acquires a pale pink colour. In fig. 15 the staining was not sufficiently prolonged to affect the chlorophyll-corpuscles.

FIG. 24.—Irregular block-like mass ( $\frac{1}{1000}$ th inch in diameter), from an olive-green specimen of *Hydra fusca*, naturally coloured green by chlorophyll, and exhibiting a small enclosed particle of colourless protoplasmic substance, which has been stained by picrocarmine.





## JOURNAL OF MICROSCOPICAL SCIENCE.

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### EXPLANATION OF PLATE XXI,

Illustrating Mrs. Ernest Hart's 'Note on the Formation of Fibrine.'

FIG. 1.—Red blood-corpuscles, isolated and fixed with osmic acid vapour, and stained with nitrate of rosanilin in absolute alcohol. The darkest-stained corpuscles are red corpuscles in their normal condition; those which are of a pale pink tint are the invisible corpuscles of Norris. Human blood.

FIG. 2.—Red blood-corpuscles, treated in the same way. A, normal red corpuscle; B, pale corpuscle; C, transparent corpuscles, beginning to send out processes. Human blood.

FIG. 3.—Pale and transparent corpuscles, sending out ramifications. A, A, A, the branches are proceeding from one point of the corpuscle only; B, B, corpuscles generally breaking down, and sending out branches in different directions. Human blood.

FIG. 4.—Group of transparent corpuscles, sending out bifurcating branches. Rabbit's blood.

FIG. 5.—Preparation of rabbit's blood, treated by "isolation," in which groups of transparent corpuscles are seen sending out tails, which in many instances are seen to bifurcate in opposite directions. A, A, normal red corpuscles; B, B, B, transparent tailed corpuscles, those at the lower edge of the drawing are observed to be sending out branches from both ends of the corpuscle.

FIG. 6.—Network of fibrine, entangling and distorting red corpuscles in its meshes. A, A, red corpuscles; B, multinucleated white corpuscle; C, pale corpuscle; D, granules; E, fibrils of fibrine. Human blood.

FIG. 7.—A band of threads of fibrine. A, A, normal red corpuscles; B, groups of granules; C, C, red corpuscles, divided by the band of fibrine. Human blood.

FIG. 8.—Preparation of human blood, showing the part the transparent corpuscles take in the formation of fibrine. A, A, normal red corpuscles; B, B, transparent corpuscles, from which threads of fibrine are seen to proceed; C, hæmotoblast of Hayem.

FIG. 9.—Preparation showing crescentic corpuscles, and corpuscles in the act of discharging their contents. A, normal red corpuscle; B, pale corpuscles; C, crescentic corpuscles; D, crescentic corpuscle with its circular rim complete; E, E, corpuscles discharging their contents.

FIG. 10.—Preparation showing the various changes of the red blood-corpuscles. A, normal red corpuscle; B, pale corpuscles; C, transparent corpuscles, three of which are seen to be sending out bifurcating branches; D, hæmotoblasts of Hayem.



## JOURNAL OF MICROSCOPICAL SCIENCE.

### EXPLANATION OF PLATES XXII, XXIII & XXIV,

#### Illustrating Mr. T. Iwakawa's Paper on "The Genesis of the Egg in Triton."

In all these plates the same letters have been employed to mark corresponding parts.

*b.* Blood-corpuscle. *f. ep.* Follicular epithelium. *g. ep.* Germinal epithelium. *g. c.* Germ-cell. *g. n.* Nucleus of the germ-cell. *g. sp.* Germinal spots. *g. v.* Germinal vesicle. *l. ep.* Lining epithelium. *l. n.* Nucleus of the lining epithelium. *n.* Nucleus of the germinal epithelium. *p. o.* Primordial ovum. *st.* Stroma. *st. n.* Nucleus of the stroma. *v. m.* Vitelline membrane. *y.* Yolk-spherules. *y. p.* Yolk-patches.

All figures were outlined by the aid of the *camera lucida*.

#### PLATE XXII.

FIG. 1.—A small piece of the ovary of an adult Triton, treated with argentic nitrate, seen from the external surface. The three strata—*germinal epithelium* (*g. ep.*), *stroma* (*st.*), and *lining epithelium* (*l. ep.*)—are distinguished by the size and colour of their nuclei—brown, yellow, and pink, respectively. Two germ-cells (*g. c.*) are seen still within the mother-cell.  $\times 450$ .

FIG. 2.—A portion of the germinal epithelium, showing a nucleus in process of division.  $\times 450$ .

FIG. 3.—A portion of the same, showing the two nuclei some time after the division. The nucleus of the germ-cell is granular.  $\times 450$ .

FIG. 4 represents a germ-cell with two nuclei still within the mother-cell, which is surrounded by six epithelial cells, the nuclei of which lie in close proximity with the central mother-cell.  $\times 450$ .

FIG. 5.—A similar case, in which the germ-cell has but one nucleus, as is usually the case at this stage.  $\times 450$ .

FIG. 6.—The germ-cell (*g. c.*) begins to project beyond the limits of the mother-cell, under the adjacent epithelial cells.  $\times 450$ .

FIG. 7.—A single mother-cell, treated with acetic acid. The nucleus (*n.*) has a peculiar form, never seen in non-proliferating cells. The expansion of the germ-cell has probably caused it to become concave on the side of contact.  $\times 450$ .

FIG. 8.—A young ovum, with germinal vesicle and yolk concrement.  $\times 165$ .

FIG. 9.—An ovum, surrounded with nuclei of the germinal epithelial cells and five germ-cells, in two of which the nuclei are in process of division. Treated with Kleinenburg's fluid. Cell limits not seen.  $\times 165$ .

## PLATES XXII, XXIII & XXIV—continued.

FIG. 10.—Three germ-cells, one of which shows a multinuclear condition. The surrounding nuclei belong to the germinal epithelium. Acetic acid.  $\times 450$ .

### PLATE XXIII.

FIG. 11.—Two cells of germinal epithelium, each possessing two like nuclei. Argentic nitrate.  $\times 450$ .

FIG. 12.—Four epithelial cells, coloured after a preparation in nitrate of silver. One cell has a nucleus in close contact with a very young germ-cell (*g. c.*).  $\times 450$ .

FIGS. 13, 14, and 15.—Sections showing the arrangement of the nuclei in the epithelial *cell-islands*. In Fig. 15 two germ-cells are seen within the epithelial layer, corresponding to the surface view in Fig. 7.  $\times 450$ .

FIG. 16 represents two germ-cells in a somewhat more advanced condition than is seen in Fig. 15.  $\times 450$ .

FIG. 17.—An epithelial island, as seen from surface of a preparation in silver nitrate.  $\times 450$ .

FIG. 18.—A young ovum, lying in the stroma, and surrounded with follicular epithelium, of which only five nuclei were to be seen.  $\times 450$ .

### PLATE XXIV.

FIG. 19.—Section of ovary wall, with an ovum ( $\cdot 24$  mm.) in position. The nucleoplasm has contracted away from the membrane and the germinal dots.  $\times 165$ .

FIG. 20.—An ovum ( $\cdot 26$  mm.) with its follicular epithelium, as seen in a fresh condition. The dark patches on one side are aggregations of yolk-spherules.  $\times 165$ .

FIG. 21.—An optical section of a portion of a follicle and ovum ( $\cdot 87$  mm.), showing the yolk-patches in little later stage of development.  $\times 450$ .

FIG. 22.—Section of an entire ovum ( $\cdot 57$  mm.), to show the arrangement and appearance of the yolk-aggregations at an early stage, and a peculiar condition of the germinal vesicle.  $\times 100$ .

FIG. 23.—A part of a section of an ovum ( $\cdot 9$  mm.), showing the yolk-patches at a time when they have come to occupy the greater portion of the ovum.  $\times 450$ .

FIG. 24.—Section of an ovum ( $1\cdot 15$  mm.) and its follicular wall soon after the limits of the yolk-patches have disappeared.  $\times 450$ .

FIG. 25.—Same ( $1\cdot 4$  mm.) at a later stage.  $\times 450$ .

FIG. 26.—Same in a nearly ripe condition.  $\times 450$ .

FIG. 27.—A germinal vesicle from an ovum measuring  $\cdot 57$  mm.  $\times 165$ .

FIG. 28.—Same from an ovum  $\cdot 82$  mm. in diameter, showing the germinal dots in process of breaking up by division.  $\times 165$ .

FIG. 29.—Same from an ovum  $1\cdot 48$  mm. in diameter, after the concentration of the granular remains of the germinal dots.  $\times 165$ .



# JOURNAL OF MICROSCOPICAL SCIENCE.

## DESCRIPTION OF PLATE XXV,

Illustrating Mr. Bower's Paper on "The Germination and Embryogeny of *Gnetum Gnemon*."

All the figures, except 1, 15, 16, 17, 18, 23, 24, and 25, were traced with camera lucida.

### Key to Lettering.

*a.* = apical cell; *ap.* = apical cone; *b. sh.* = bundle sheath; *c.* = cotyledon; *cot. bun.* = cotyledonary bundle; *f.* = feeder; *hy. st.* = hypocotyledonary stem; *lat.* = latex; *l. rt.* = lateral root; *pl. l.* = plumular leaf; *pr. ph.* = primary phloem; *pl. st.* = plumular stem; *pr. xy.* = primary xylem; *r.* = radicle; *rt.* = root; *s.* = suspensor.

### A Species of *Gnetum* from Chittagong.

FIG. 1 ( $\times 4$ ).—Groups of suspensors from the cavity in the endosperm.

FIG. 2 ( $\times 70$ ).—Single suspensory tube from the same.

### *Gnetum Gnemon*.

FIG. 3 ( $\times 175$ ).—Young embryo, external view.

FIG. 4 ( $\times 175$ ).—Young embryo, optical section.

FIG. 5 ( $\times 175$ ).—Embryo, optical section.

FIG. 6 ( $\times 175$ ).—Young embryo *in situ*, external view.

FIG. 7 ( $\times 175$ ).—Ditto.

FIG. 8 ( $\times 175$ ).—Older embryo, longitudinal section.

FIG. 9 ( $\times 175$ ).—Older embryo, longitudinal section, the peripheral cells near the apex dividing both parallel and perpendicular to the surface.

FIGS. 10—12 ( $\times 20$ ).—Older embryos in longitudinal section, showing the development of the feeder.

FIG. 13 ( $\times 325$ ).—Apex of young embryo, before the development of the feeder.

FIG. 14 ( $\times 175$ ).—Apex of stem, from an old plant: the first divisions preparatory to the formation of the glands may be seen in the young leaf.

FIG. 15 ( $\times 2$ ).—Embryo before protrusion of the radicle.

FIG. 16 ( $\times 5$ ).—Longitudinal section of feeder: abnormal case, showing the effect of pressure on its apex.

FIG. 17 ( $\times$  about  $1\frac{1}{2}$ ).—Seedling after the hypocotyledonary stem has left the seed.

FIG. 18 (nat. size).—Seedling, ten days above ground.

PLATE XXV—*continued*.

- FIG. 19 ( $\times 20$ ).—Medium longitudinal section of apex of young plant, showing the glands covering the young organs in the bud.
- FIG. 20 ( $\times 20$ ).—Proliferation of the suspensor, resulting in the formation of numerous embryos.
- FIGS. 21 and 22 ( $\times 175$ ).—Early stages of a similar proliferation.
- FIG. 23 ( $\times 10$ ).—Transverse sections through the seedling.  
*a*, at base of the cotyledons; *b*, at some distance below *a*. Bundles ( $\times$ ) result from the fusion of two of the cotyledonary bundles.
- FIG. 24 (*a*—*f*  $\times 10$ , *g*  $\times 5$ ).—Sections *a*—*f*, transverse; *g*, longitudinal, to show the relation of the bundle systems of the root and stem to one another, and to the feeder.
- FIG. 25 ( $\times 20$ ).—Transverse section of the feeder near to the hypocotyledonary stem.
- FIG. 26 ( $\times 325$ ).—Young sclerenchyma cells, seen in longitudinal section of the young stem. Each cell with two nuclei.
- FIG. 27 ( $\times 550$ ).—From longitudinal section of bud of old plant, showing perforation of wall and fusion of contents of two laticiferous cells.
- FIG. 28 ( $\times 550$ ).—Longitudinal section of wall of a laticiferous vessel seen obliquely, and showing the sac-like outgrowths encroaching on the cavity of the neighbouring cells.
- FIG. 29 ( $\times 550$ ).—Young laticiferous cells from longitudinal section of an old bud; one of them has two nuclei.
- FIG. 30 ( $\times 550$ ).—Longitudinal section passing through young laticiferous vessel, transverse wall surrounded by highly refractive mass. (cf. text.)

## JOURNAL OF MICROSCOPICAL SCIENCE.

### EXPLANATION OF FIGURES OF PLATE XXVI,

#### Illustrating Dr. Klein's Memoir on the "Organ of Jacobson in the Dog."

FIGS. 1, 2, 3, 4, 5, 6, 7, and 8, are outline drawings of successive transverse sections through the parts containing the canal of Stenson and the anterior portion of the organ of Jacobson of one side, very slightly magnified.

The meaning of the letters is:—*b*. Bone. *l*. Middle line. *c*. Cartilage. *o* in fig. 1. Opening or mouth of Stenson's canal. *p*. Surface of palate. *pp* in figs. 1 and 2. Papilla at the mouth of Stenson's canal. *S*. Stenson's canal. *J*. Jacobson's organ.

In Figs. 7 and 8, in addition:—*f*. Lower nasal furrow. *m*. Mucous membrane covering the nasal septum. *sc*. Cartilage of nasal septum.

FIGS. 9, 10, and 14 are copies of photographs taken by my friend Dr. George Maddox, to whom I am greatly indebted. The individual parts are understood by a reference to figs. 11 and 15.

Fig. 14 is a representation of the organ of Jacobson of the right side of fig. 9, but under a higher power.

In Fig. 11 the meaning is of:—1. Upper bone lamella of the crista nasalis. 2. The middle bone or the intermaxillary bone. 3. The lower portion continuous with the palatine plate of the superior maxilla. *c*. Jacobson's cartilage. *J*. Jacobson's organ. *sc*. Septal cartilage. *m*. Mucous membrane of the nasal septum.

FIGS. 12 and 13 are representations of successive transverse sections through the posterior extremity of the organ of Jacobson. *b*. Bone of the crista nasalis. *c*. Jacobson's cartilage. *J*. Jacobson's organ. *m*. Mucous membrane of the nasal septum. *sc*. Septal cartilage.

FIG. 15.—From a transverse section through the organ of Jacobson in about its middle. Magnifying power about 50. *c*. Inner boundary line of Jacobson's cartilage. *b*. Transverse sections through blood-vessels. *g*. Serous glands. *n*. Transverse sections through branches of the olfactory nerve. *l*. Lateral wall of organ of Jacobson. *m*. Median wall of same. *s*. Septum between lateral wall of organ of Jacobson and the mucous membrane of nasal septum.

FIG. 16.—From a vertical section through the sensory epithelium of the median wall. Magnifying power about 750. *c*. Superficial cuticle. *e*. "Epithelial" cells. *s*. "Sensory" cells. *b*. Deepest layer of epithelial cells.





Fig. 1

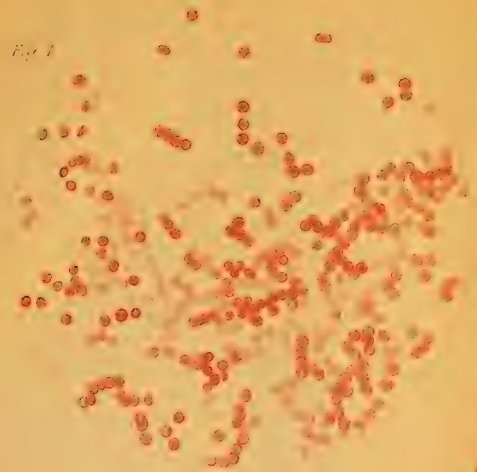


Fig. 2



Fig. 3

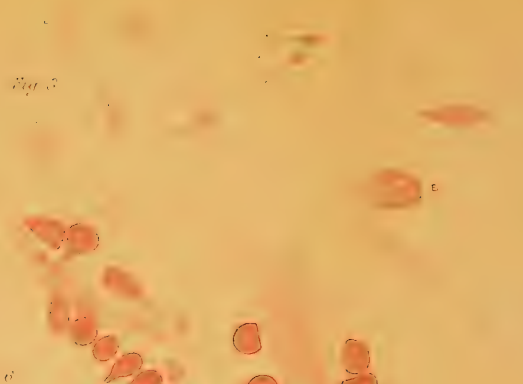


Fig. 4



Fig. 5

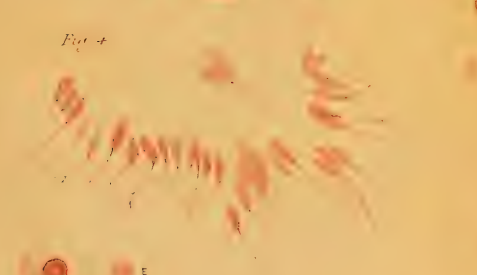


Fig. 6

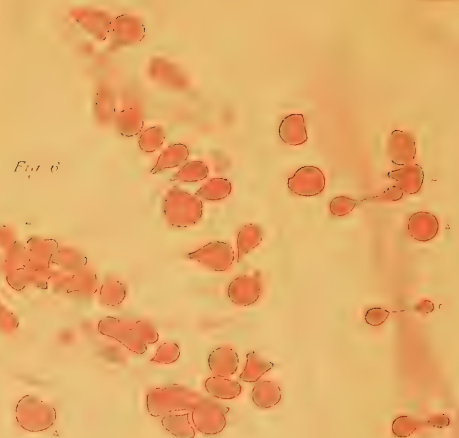


Fig. 7

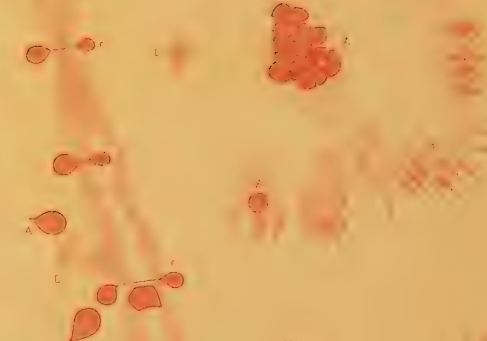


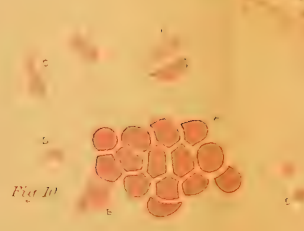
Fig. 8



Fig. 9



Fig. 10







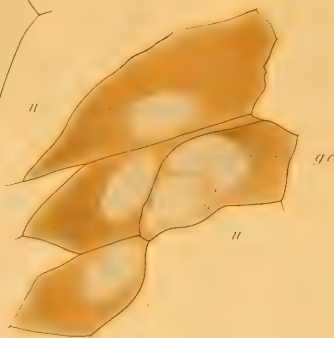




*Fig. 11. (x<sup>200</sup>)*



*Fig. 12. (x<sup>200</sup>)*



*Fig. 13. (x<sup>200</sup>)*

*g. Ep.*

*n*

*Fig. 14. (x<sup>200</sup>)*

*g Ep*

*Fig. 15. (x<sup>200</sup>)*

*g n*

*n*

*Fig. 17. (x<sup>200</sup>)*

*g Ep*

*n*

*g c*

*g Ep.*

*g c*

*g c*

*n*

*Fig. 16. (x<sup>200</sup>)*

*g. Ep.*

*Sl*

*l Ep.*

*Fig. 18. (x<sup>200</sup>)*

*l Ep. g Ep.*

*Sl.*

*l Ep*

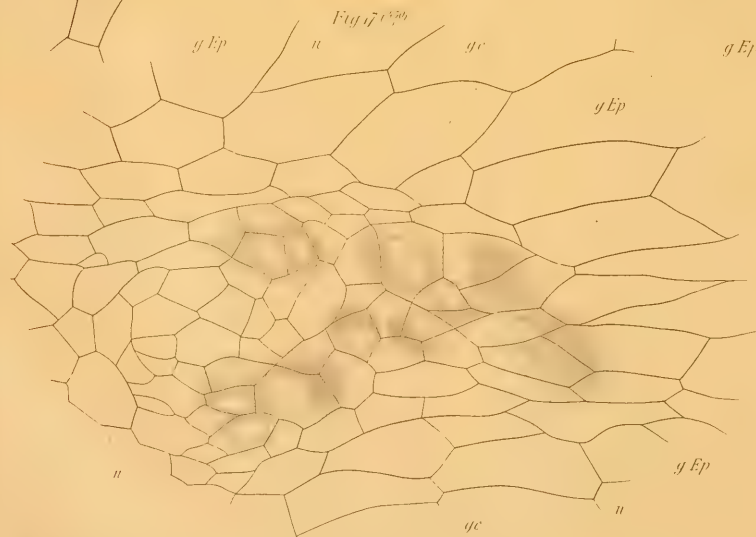














Fig. 1.



Fig. 3.



Fig. 5.

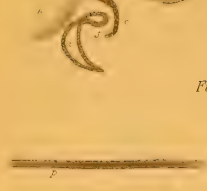


Fig. 7.

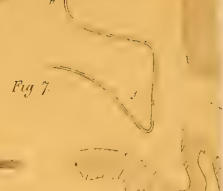


Fig. 2.



Fig. 4.



Fig. 6.

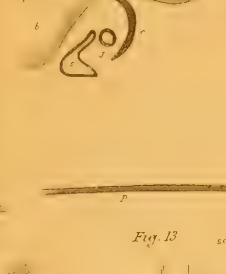


Fig. 13.

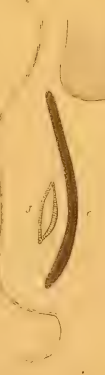


Fig. 11.



Fig. 8.



Fig. 9.



Fig. 15.



Fig. 10.



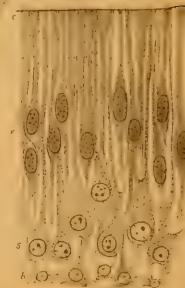
Fig. 12.



Fig. 14.



Fig. 16.







## JOURNAL OF MICROSCOPICAL SCIENCE.

### EXPLANATION OF PLATE XXVII,

Illustrating Dr. Horst's Paper on "The Development of the European Oyster (*Ostrea edulis*, L.)."

FIG. 1.—An egg in segmentation, consisting of a large hypoblastic sphere and several epiblastic cells.

FIG. 2.—Surface view of a later stage, with two hypoblastic cells.

FIG. 3.—Embryo viewed from the side, with beginning invagination (gastrula).

FIG. 4.—Optical section through a more advanced stage, with the hypoblastic invagination and the first apparition of the shell-gland—*ec*, epiblast; *en*, hypoblast; *o*, blastopore; *sh*, shell-gland.

FIG. 5.—An older embryo viewed from the side—*v*, foot.

FIG. 6.—An optical section through the same stage—*me*, mesoblast; *d*, archenteron.

FIG. 7.—Stage one day older, viewed from the front side, with blastopore.

FIG. 8.—Optical section through the same stage.

FIG. 9.—Optical section through an embryo, one day older, with velum, stomach and shell, *s*.

FIG. 10.—A later stage, viewed from the side, with more developed shell.

FIG. 11.—More advanced larva, with the epiblastic thickening in the midst of the velar area—*a*, anus; *e*, intestine; *m*, stomach; *sl*, œsophagus; *tp*, cephalic thickening.

FIG. 12.—Older larva with a double præoral ring of cilia, nerve ganglion, hepatic sacs and muscles—*ds*, dorsal longitudinal muscle; *vs*, ventral longitudinal muscle; *sp*, adductor muscle; *l*, hepatic sac; *mh*, pallial cavity.

## JOURNAL OF MICROSCOPICAL SCIENCE.

### EXPLANATION OF PLATES XXVIII & XXIX,

Illustrating Mr. H. Marshall Ward's Memoir on "The Morphology and Life-history of a Tropical Pyrenomycetous Fungus."

FIG. 1.—Two leaves of *Jasminum pubescens* affected by the epiphytal mycelium; *a* was still young, and preserved its green colour; *b* was turning yellow at the affected spots. Natural size.

FIG. 2.—A small specimen of the fungus as seen with a low power; the round bodies are "fruits" in various stages of development.

FIG. 3.—One of the above "fruit-bodies" more highly magnified (Zeiss D). It is ripe, and has become opened by radial slits above, disclosing the asci within.

FIG. 4.—A similar "fruit-body" seen in vertical section. Zeiss D.

FIG. 5.—Germinating spore, showing early development of *haustorium* from the young mycelium. Zeiss J.

FIG. 6.—Small portion of older mycelium, showing *haustoria* from the short lateral branches. Zeiss J.

FIG. 7.—Small portion of old mycelium, showing breaking up of the lateral branches, each portion being capable of vegetative reproduction Zeiss E.

FIG. 8.—Sections through upper parts of leaf on which the mycelium is well developed, and showing the *haustoria* penetrating into the cells. The upper figures show cross sections of the hyphæ; in the lower one the razor has partially cut a radial branch longitudinally. Zeiss D and J.

FIG. 9.—Portion of mycelium on which the "fruit-bodies" are commencing to appear. Zeiss D.

FIG. 10.—Part of the above more highly magnified. Zeiss J.

FIG. 11.—Early stage of a young "fruit-body." Two septa have appeared in one cell of the hypha, and lateral outgrowths are spreading to form the disc. Zeiss E.

FIG. 12.—More advanced condition of similar "fruit-body;" the young disc is formed. Zeiss E.

FIG. 13.—An advanced state of the disc seen from above, and under which the ascogenous tissue is beginning to form. Zeiss E.

FIG. 14.—Vertical section of a young disc some distance from the median line; the space beneath is becoming filled with delicate hyphæ. Zeiss E.

FIG. 15.—Similar section (slightly oblique) nearer the median line of an older disc. The dark shading represents the cellular walls without details; the cavity is filled with delicate hyphæ radiating from the walls. Zeiss E.

EXPLANATION OF PLATES XXVIII & XXIX.—*Continued.*

FIG. 16.—Vertical section through a nearly ripe “fruit-body” about the median line; several asci are far advanced, others and younger ones are being produced among the delicate filaments. Zeiss E.

FIG. 17.—Very thin median vertical section through a fruit-body at a stage somewhat younger than fig. 16; among the hyphæ filling up the space beneath is the young coil of ascogenous hyphæ, the cells of which are filled with fine grained protoplasm. Zeiss J.

FIG. 18.—Ascogenous hyphæ bearing asci in various stages; from vertical sections about the stage represented in fig. 16. Zeiss J.

FIG. 19.—Filaments and young ascogenous hyphæ obtained from the inside of a fruit-body by dissection; about the same age as fig. 16. Zeiss J.

FIG. 20.—Portions of the cellular disc bearing fine branched filaments which radiate into the cavity; about the same stage as the last. Zeiss J.

FIG. 21.—Ascogenous hyphæ and young asci obtained from the inside of a fruit-body at the same stage. In one ascus the protoplasm has already begun to divide; contents contracted by glycerine. Zeiss J.

FIG. 22.—Portion of vertical section through a young fruit-body, showing relations of the parts figured; a young ascus has arisen from the mass on the floor. Zeiss J.

FIG. 23.—An ascus somewhat more advanced than in fig. 22; the protoplasm has partially divided into four. Zeiss J.

FIG. 24.—Similar preparations in glycerine; in one case the protoplasm, mostly divided into a tetrad, is pressed out from the young ascus.

FIG. 25.—Portion of the mycelium on which two tufts of the “sporidia” bearing filaments have arisen; the filaments and “sporidia” are enveloped in jelly. Zeiss D.

FIG. 26.—Vertical sections through the points of origin of the tufts; the curved spore-like bodies are produced from the ends of the filaments. Zeiss D.

FIG. 27.—“Sporidia,” and the filaments producing them more highly magnified. Zeiss J.

FIG. 28.—Portion of mycelium with two of the tufts. Zeiss J.

FIG. 29.—One of the tufts with numerous minute granules embedded in the jelly. Zeiss E.

FIGS. 30 and 31.—Similar tufts from the lateral lobes which produce the discs; here, as in figs. 28 and 29, no “sporidia” are as yet abstracted off.

FIG. 32.—Larger branches of the mycelium, the cells of which retain their pale colour, and separate as toruloid individuals. Zeiss J.

## JOURNAL OF MICROSCOPICAL SCIENCE.

### EXPLANATION OF PLATE XXX,

#### Illustrating Mr. J. E. Blomfield's Paper on "The Thread-cells and Epidermis of Myxine."

FIG. 1.—Section of the body-wall of *Myxine glutinosa*, showing—*a*, epidermis; *b*, dermis; *c*, subcutaneous fatty tissue; *d*, club-cells or yellow-staining cells; *e*, spider-cells or granular cells of Foettinger. Picric acid preparation stained with picro-carmin.

FIG. 2.—Various cells from the epidermis. Series *a*, goblet cells; *b*, cells from the basal layer of the epidermis, showing expanded bases of the cells; *c*, cells from the intermediate layers.

FIG. 3.—"Spider" cells (granular cells of Foettinger in *Petromyzon*) from the epidermis.

FIG. 4.—"Yellow" cells (club-cells of Foettinger) from the epidermis.

FIG. 5.—Portion of the contents of a lateral mucus-gland, showing the "thread" cells and stroma of "spider" cells.

FIG. 6.—Thread cells from a gland, showing their mode of staining with picrocarmin.

FIG. 7.—Thread cells, showing the mode of winding of the thread—*a*, *b*, *c*, different views of same cell under different foci.

FIG. 8.—Section through a young gland, showing continuity of the gland cells with the epidermic cells and the origin of the thread cells from the epidermic cells. *m*, muscular investment of the gland sac; *d*, club-cells or yellow-staining cells of the epidermis; *e*, granular cells stained by carmine, identical with the 'spider-cells' of Fig. 1. Alcohol was used in preserving this specimen; picric acid in the case of Fig. 1.

FIG. 9.—Development of a thread cell, showing gradual deposition of the yellow-staining material.

FIG. 10.—Mucus from the surface of a *Myxine*, showing the thread cells unwinding, threads, mucus cells, and epidermic cells.



# JOURNAL OF MICROSCOPICAL SCIENCE.

## DESCRIPTION OF PLATE XXXI,

Illustrating Prof. Haddon's "Notes on the Development of Mollusca."

*The outlines of all the figures were traced with the camera lucida.*

*a.*, anus; *au.*, auditory involution; *bl.*, blastopore; *c. c.*, ciliated epithelium of foot; *e.*, eye; *ep.*, epiblast; *e. s.*, egg-shell; *ft.*, foot; *hy.*, hypoblast; *i.*, intestine; *i. o. c.*, infra-oral circle of cilia; *l.*, liver (with remainder of yolk); *m.*, mesoblast; *m. c.*, mantle-cavity, within which the velum can be retracted; *mtk.*, mouth; *œ.*, œsophagus; *op.*, temporary operculum; *opt.*, optic involution; *ot.*, otocyst; *p.*, pigment; *p. c.*, polar cells; *p. g.*, pedal ganglion; *p. k.*, provisional kidney; *r.*, rectum; *r. m.*, retractor muscle; *s.*, shell; *s. g.*, supra-œsophageal ganglion; *st.*, stomach; *v.*, velum; *y.*, yolk; *y. c.*, yolk-cells.

Fig. 1. *Elysia viridis*. Figs. 2—5. *Piona* sp.? Figs. 6—11, *Janthina fragilis*. Figs. 12—14. *Purpura lapillus*. Figs. 15—17. *Murex erinaceus*?

Fig. 1.—Gastrula formation, with elongated blastopore.  $\times 320$ .

Fig. 2.—Gastrula, blastopore still slit-like.  $\times 320$ . Compare Lankester, 'Phil. Trans.,' 1875, Plate 9, fig. 5, and Plate 10, fig. 2.

Fig. 3.—Young veliger, with boat shaped larval-shell, showing the two cilia (*x*) in the centre of the velum. Note the temporary larval shell and operculum.

Fig. 4.—Oral view of velum of an older embryo, showing the velar groove with the superior motor cilia and the infra-oral circle of cilia above the mouth. The two central cilia (*x*) are seen in the distance.  $\times 630$ .

Fig. 5.—Three-quarter aboral view of an old veliger, with ovoid shell, showing the velar groove and central pair of cilia.

Fig. 6.—Ovum, with the four primitive epiblast cells segmenting off from the four primary segmentation spheres.

Fig. 7.—A similar ovum with the process completed.

Fig. 8.—The succeeding stage.

Fig. 9.—Solid blastula with an epiblastic cap.

Fig. 10.—The cap of epiblast cells growing round the large yolk cells, thus forming a gastrula by epibolè—at the junction of the epiblast with the yolk cells, *i. e.* the lip of the blastopore—are two mesoderm cells (*m*).

Fig. 11.—Right-side view of a well-developed veliger, with all the larval organs; aboral to the ciliated anus (*a*) is the large violet-coloured pigment mass. The shell itself is of a light madder tint. Note the temporary operculum (*op.*). Compare Lankester, 'Phil. Trans.,' 1875, Plate 8, fig. 28.

Fig. 12.—Left-side view of a young veliger with rudimentary shell (*s.*), showing the surface organs.  $\times 90$ .

Fig. 13.—Transverse section through anterior end of above, showing the paired patches of thickened and proliferating epiblast in the velum and foot.  $\times 90$ .

Fig. 14.—Enlarged view of a portion of above, showing the formation of the supra-œsophageal ganglion.  $\times 320$ .

Fig. 15.—Transverse section through the foot, showing the origin of the pedal ganglion by the side of the already formed otocyst. Above the ciliated epithelium of the foot is a section of a portion of the œsophagus.

Fig. 16.—Section through the optic involution; the black pigment of the retina is already being formed at the fundus.

Fig. 17.—Section through the auditory involution.

## JOURNAL OF MICROSCOPICAL SCIENCE.

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### EXPLANATION OF PLATE XXXII,

Illustrating Mr. Vincent Harris's Note "On Pacinian Bodies."

FIG. 1.—Cells of the pancreas of a dog, with adjacent Pacinian corpuscle. Zeiss, obj. A, oc. 3.

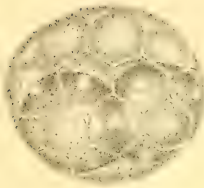
FIG. 2.—Ganglion cells, adjacent to the Pacinian corpuscle drawn in Fig. 1.

FIG. 3.—Section through a mass of Pacinian corpuscles, seen in the middle pad of a kitten's fore-paw. Zeiss, obj. D, oc. 2.

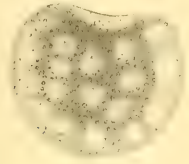
*Fig. 1.*



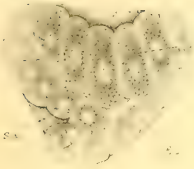
*Fig. 2.*



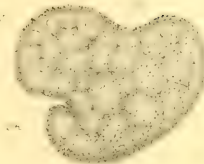
*Fig. 3.*



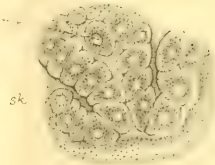
*Fig. 4.*



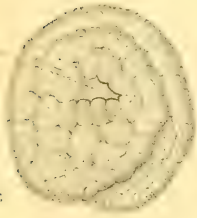
*Fig. 5.*



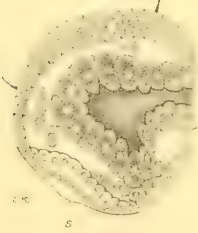
*Fig. 6.*



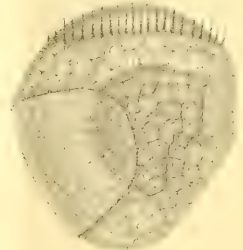
*Fig. 7.*



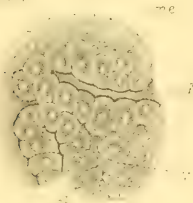
*Fig. 9.*



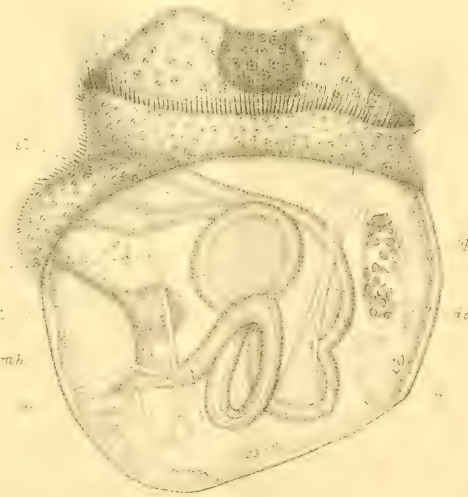
*Fig. 10.*



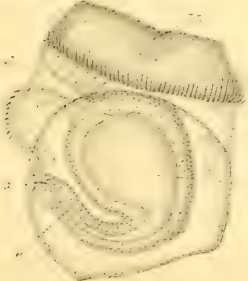
*Fig. 8.*



*Fig. 12.*



*Fig. 11.*



## JOURN

EN

### Illustrating

FIG. 1.—Ce  
puscle. Zeiss.

FIG. 2.—Ga  
Fig. 1.

FIG. 3.—Se  
middle pad of



Fig 1

b

Fig 2

Fig 11.

Fig 12.

Fig 13.

a

Fig 3.

Fig 4.

Fig 9.

Fig 16.

Fig 15.

Fig 14.

Fig 17.

Fig 5.

Fig 7

Fig 8.

Fig 6.

Fig 10.

Fig 18.



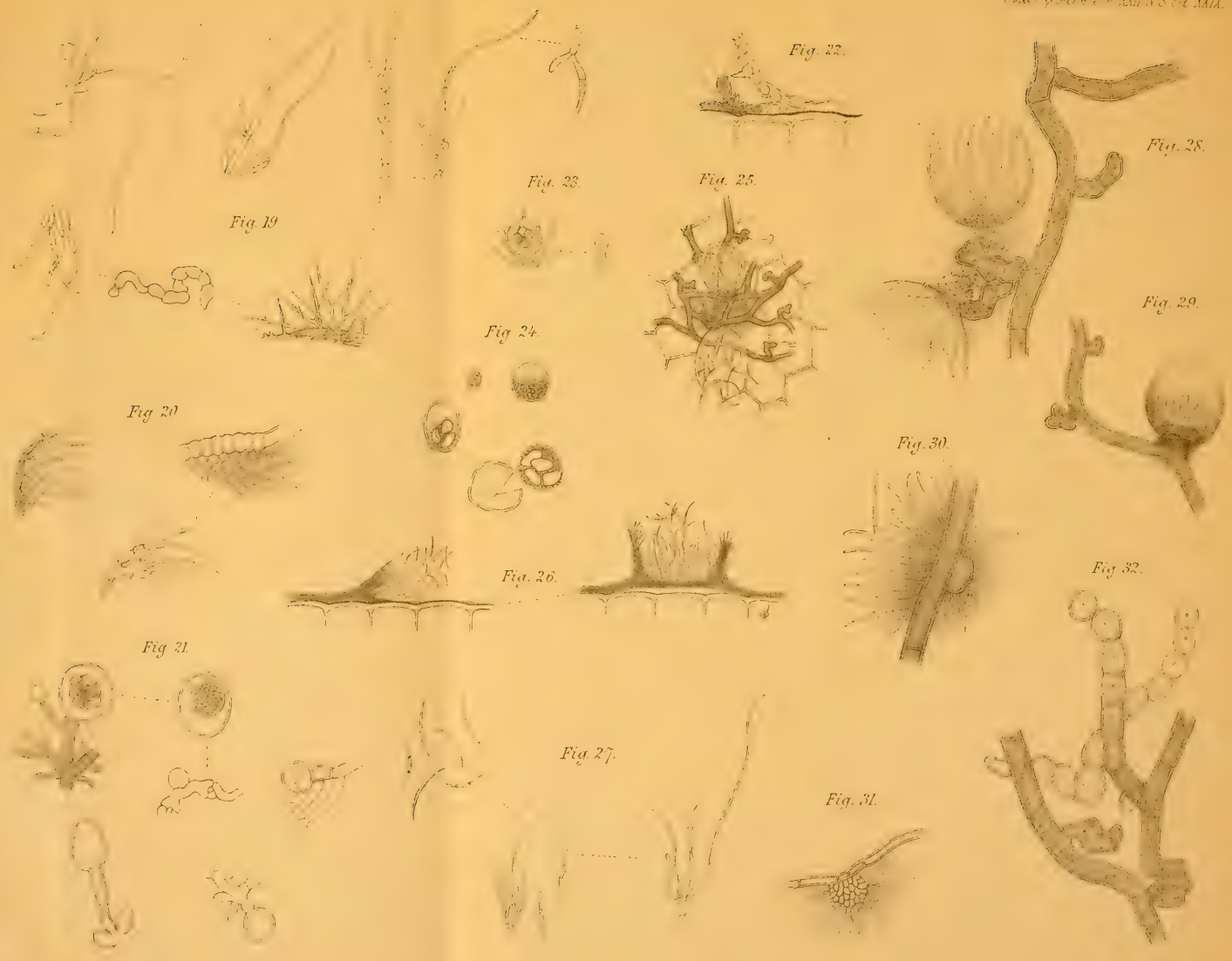
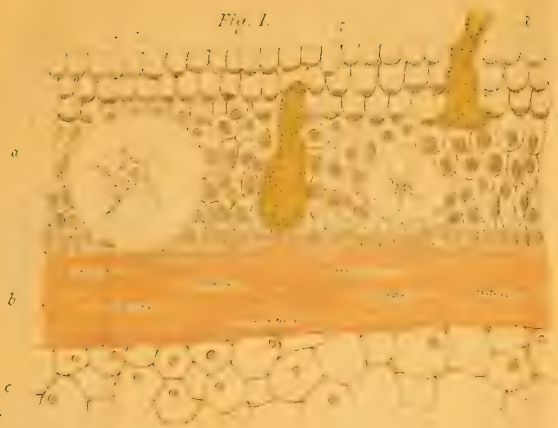






Fig. 1.



a

a

b

c

Fig. 2.

b

Fig. 3.

Fig. 4.

Fig. 6.

Fig. 8.

Fig. 5.

Fig. 7.

Fig. 9.

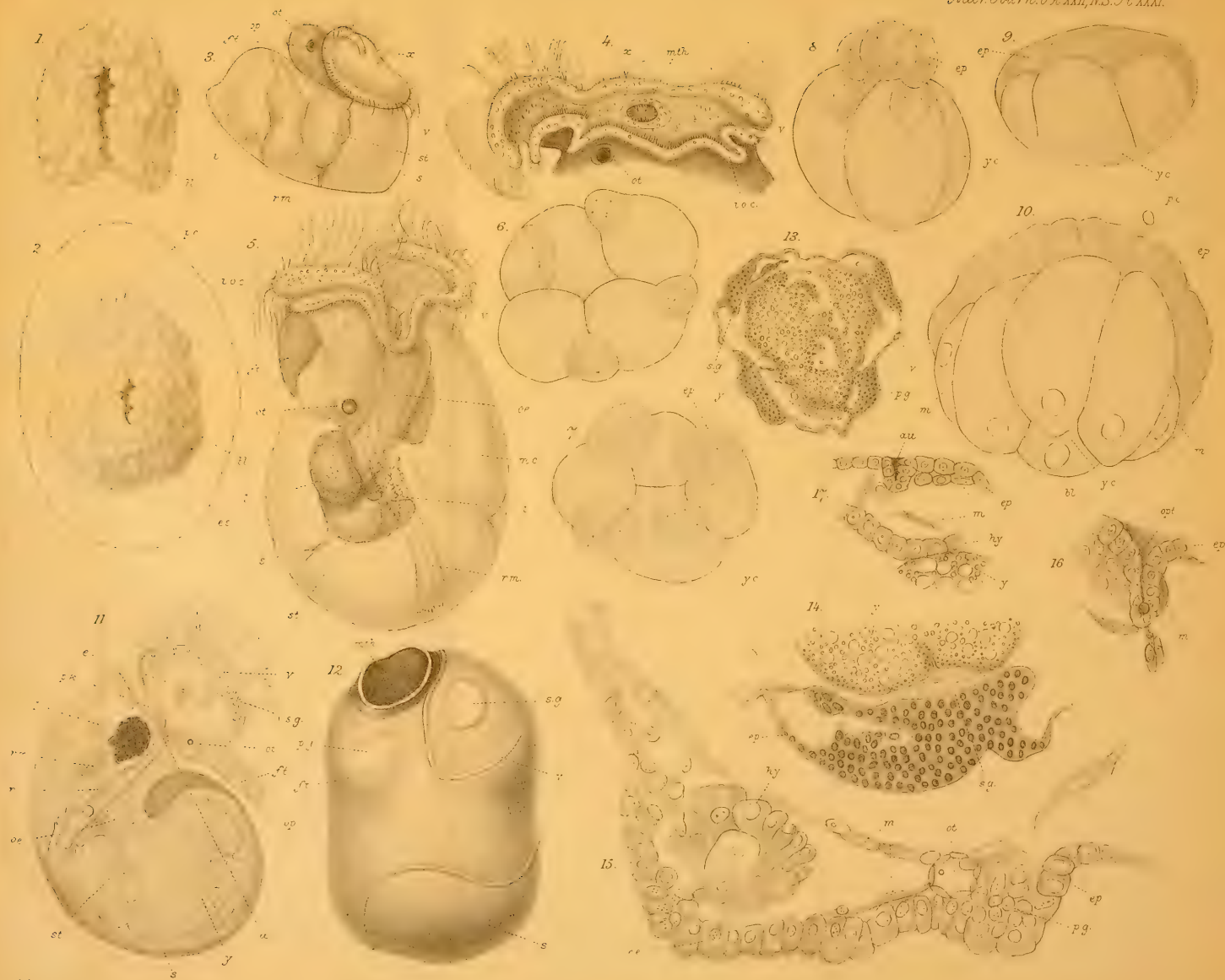
Fig. 10.

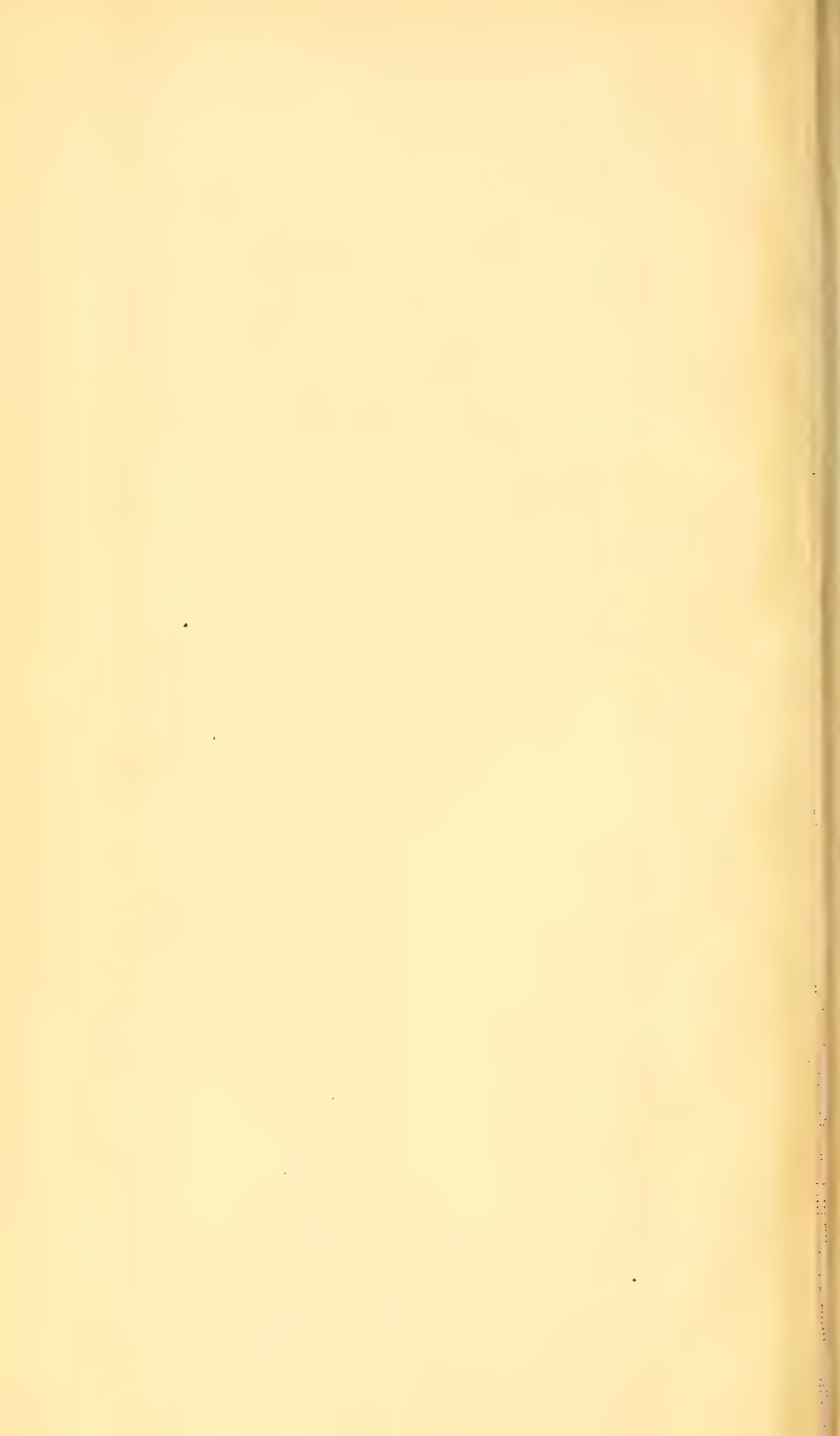
a

b

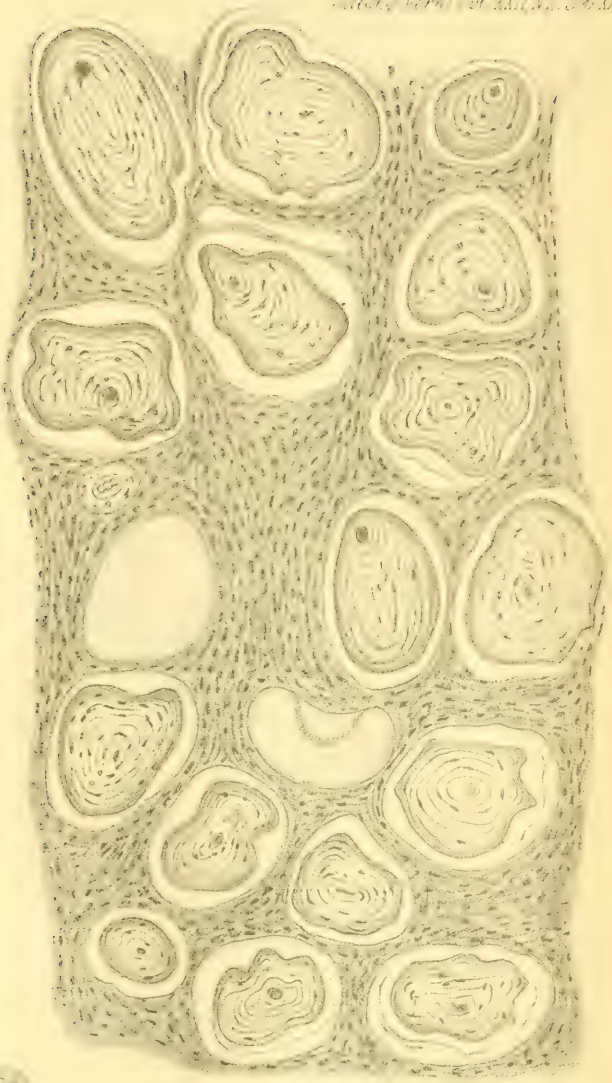
c





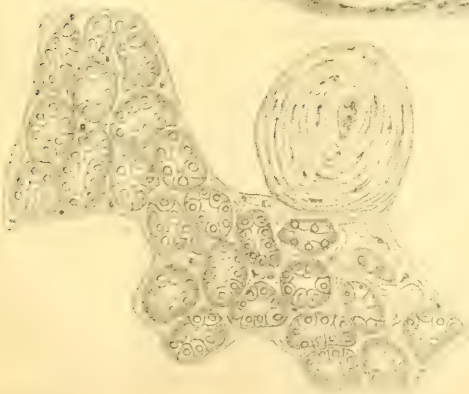






*Fig. 3.*

*Fig. 1.*



*Fig. 2.*





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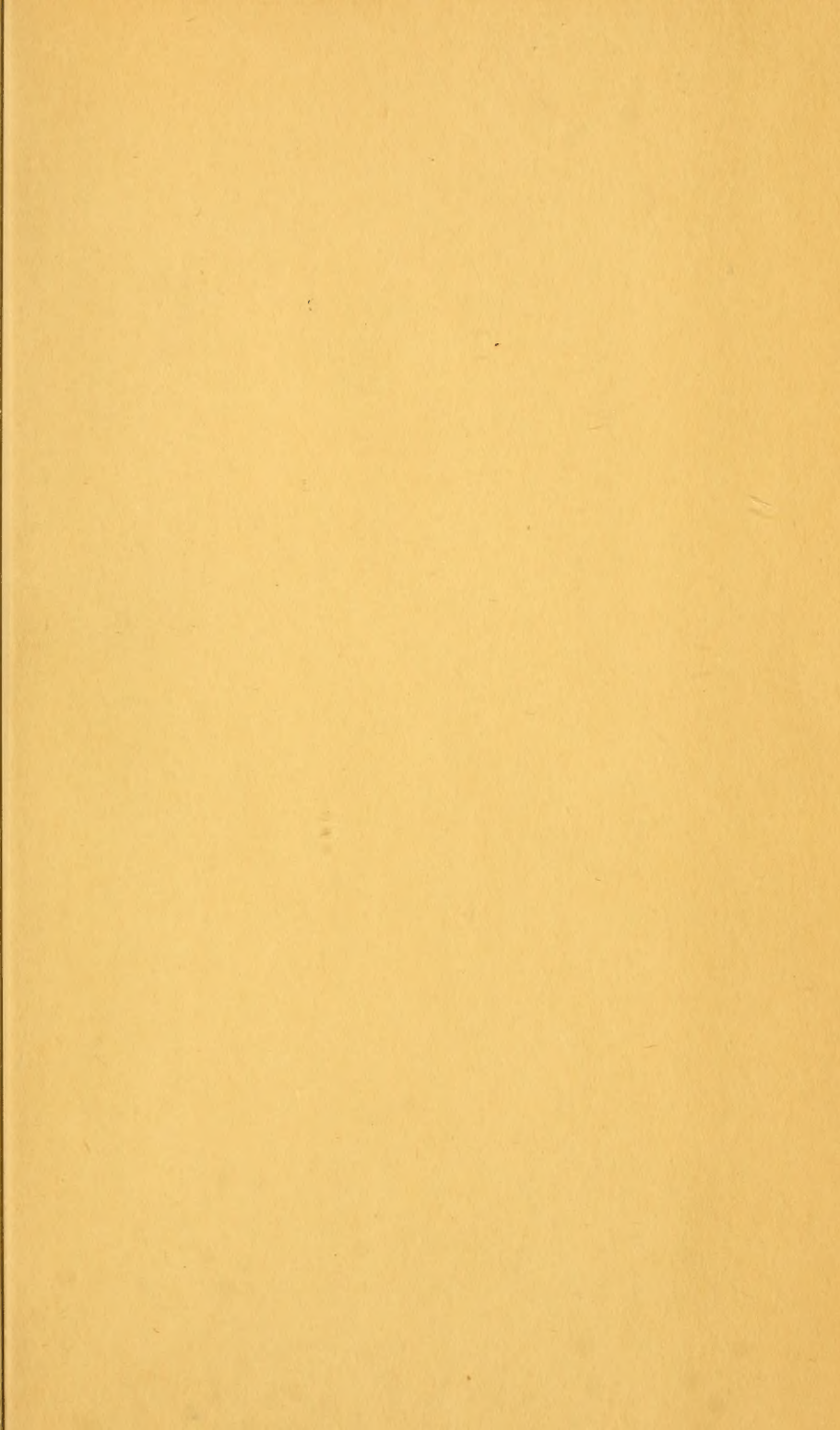
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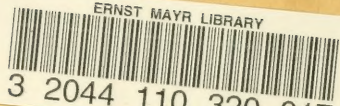








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